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UTILIZATION OF CALCIUM BY RATS ON HIGH PROTEIN–LOW CALCIUM AND HIGH CARBOHYDRATE–LOW CALCIUM DIETS

EFFECT OF SUPPLEMENTARY VITAMIN D

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The work which we shall describe was planned to test the hypothesis that a high carbohydrate diet causes poor retention of calcium and thereby an increase in susceptibility to dental caries, especially in adolescent persons on a suboptimal intake of calcium and vitamin D.

Two groups of young rats were fed a low carbohydrate–high protein diet for a year, one with, and the other without, added vitamin D. Two other groups were fed a high carbohydrate–low protein diet, one with, and the other without, added vitamin D. The percentages of the other constituents of the diets were the same for all of the groups and were adequate for nutrition and growth except with regard to calcium, which was low.

The results showed that high dietary carbohydrate does not diminish retention of calcium but, on the other hand, increases the effectiveness of vitamin D in assisting the retention of calcium.

EXPERIMENT

Animals.—Twenty-four male albino rats, weighing about 100 Gm. each, were selected. These were divided into four groups and so arranged that the total weight of each group was approximately the same. Each group was kept in a cage provided with a coarse screen bottom, and shaded at all times from bright light. The McCollum type of food dish [1] was used, so that the spilling of food by the rats was avoided. The ration was eaten ad libitum.

Diets.—The composition of the diets is given in table 1 and that of the salt mixtures in table 2. It was necessary to use two slightly different salt mixtures in order to compensate in the high carbohydrate diet for the extra calcium and phosphorus contained in the larger percentages of casein used in the high protein diet. The percentage of the inorganic constituents in the finished rations except for calcium was substantially the same as would be given by 4 per cent of the Osborne and Mendel [2a] salt mixture or 3.5 per cent of an entirely inorganic mixture previously described.[2b] By analysis both diets contained 18 mg. of calcium per hundred grams of diet. By calculation the high protein diet contained 290 mg. of phosphorus, and the high carbohydrate diet 280 mg. of phosphorus per hundred grams of diet. The phosphorus/calcium ratio of both diets was therefore approximately 16/1. Low calcium diets were chosen in order to render the relative effects of the vitamin D deficiencies more pronounced.

Both salt mixtures were practically neutral. The acid value of the two diets was therefore calculated as the sum of the acid values of the casein and the yeast. Dried brewers' yeast

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1. This dish is made by the George H. Wahmann Manufacturing Company, of Baltimore.

2. (*a*) Osborne, T. B., and Mendel, L. B.: J. Biol. Chem. **37**:557, 1919. (*b*) Wesson, L. G.: Science **75**:339, 1932.

on the basis of the analysis of its ash [3] is equivalent to 28 cc. of tenth-normal acid per 4 Gm. of yeast. The total acid value of the high protein diet with phosphoric acid considered as dibasic was 349 cc., and that of the high carbohydrate diet was 135 cc., of tenth-normal acid per hundred grams of diet.

Since yeast contains approximately 50 per cent protein,[3] the total protein content of the two diets was 38 per cent and 14 per cent, respectively.

The calculated available heat values of the two diets on the usual basis (protein and carbohydrate 4 calories, and fat 8.9 calories, per Gm.) was 410 calories per hundred grams of diet.

Method of Analysis for Excreted Calcium.—The twenty-four hour excreta of each of the four groups of rats were collected biweekly in a paraffined metal tray. Cheesecloth was placed beneath each cage to catch the occasional droppings that were deflected outside the trays. The feces and the water rinsings of the trays were dried separately and then combined for treatment with nitric acid. For this purpose 20 cc. of concentrated nitric acid was added in portions to each combined sample in a covered 600 cc. pyrex beaker heated on the steam

TABLE 1.—*High Protein–Low Calcium and High Carbohydrate–Low Calcium Diets*

	Rat Group I	Rat Group II	Rat Group III	Rat Group IV
	High Protein Diet		High Carbohydrate Diet	
	With D	Without D	With D	Without D
Dextrin, white, %	53.9	53.9	77.8	77.8
Casein, "vitamin-free," % *	36.0	36.0	12.0	12.0
Salt mixture A (table 2), %	2.10	2.10	0	0
Salt mixture B (table 2), %	0	0	2.19	2.19
Yeast, dried (Harris), %	4.0	4.0	4.0	4.0
Butter fat, %	4.0†	4.0†	4.0†	4.0†
Vitamin D (viosterol), U. S. P. units	272	0	272	0
Carotene, daily supplement, mg.	0.025	0.025	0.025	0.025

* The casein was prepared by the Borden Company, 350 Madison Avenue, New York.
† Four per cent butter fat is equivalent to 1.6 to 6 international units of vitamin D per hundred grams of diet as calculated from "The Newer Knowledge of Nutrition," fifth edition, by E. V. McCollum, E. Orent-Keiler and H. G. Day (New York, the Macmillan Company 1939).

TABLE 2.—*Salt Mixtures Used in the High Protein–Low Calcium and the High Carbohydrate–Low Calcium Diets of Table 1*

	Salt Mixture A for High Protein Diet	Salt Mixture B for High Carbohydrate Diet
Sodium chloride (NaCl), %	17.5	16.8
Potassium chloride (KCl), %	48.3	27.0
Dipotassium hydrogen phosphate (K_2HPO_4), %	....	22.5
Magnesium sulfate ($MgSO_4.7H_2O$), %	30.9	29.7
Calcium lactate ($Ca[C_3H_5O_3]_2.5H_2O$), %	....	0.8
Iron lactate ($Fe[C_3H_5O_3]_2.3H_2O$), %	3.1	3.0
Manganese sulfate (anhydrous) ($MnSO_4$), %	0.032	0.032
Potassium alum ($KAl[SO_4]_2.12H_2O$), %	0.0144	0.0144
Copper sulfate ($CuSO_4.5H_2O$), %	0.064	0.064
Sodium fluoride (NaF), %	0.091	0.091
Potassium Iodide (KI), %	0.008	0.008

bath. After all of the acid had been added, heating was continued for one hour; the acid was then diluted with 15 cc. of water and allowed to cool. After filtering the acid solution into a 50 cc. glass-stoppered volumetric flask, it was made up to volume with the washings of the filter. Ten cubic centimeter samples were used for the analyses.

Calcium was determined in duplicate by the method of Fiske and Logan [4] except that the ignited calcium oxide was redissolved in 2 cc. of hot normal nitric acid, rinsed back into the test tube used for the precipitation of the oxalate and then reprecipitated. This added operation destroyed residual organic matter which seemed to interfere with the accuracy of the analyses.

Method of Analysis of the Diets for Calcium.—The diets were oxidized and analyzed by a similar procedure, with 10 Gm. samples being used in the preparation of the 50 cc. volumes used for the duplicate determinations.

3. Hawk, P. B.; Smith, C. A., and Holder, R. C.: Am. J. Physiol. **48**:199, 1919.
4. Fiske, C. H., and Logan, M. A.: J. Biol. Chem. **93**:211, 1931.

Method of Analysis of the Bones and Teeth for Calcium.—The air-dried leg bones were ground, extracted with alcohol and ether and then heated two hours at 100 to 110 C. The crowns of the molar teeth and the shafts of the incisors were similarly treated. Approximately 0.1 Gm. samples were weighed and ignited over a flame and then in the muffle furnace. The ash was weighed and dissolved in hydrochloric acid and the calcium precipitated as in the analyses for excreted calcium except that the total calcium oxide of the sample was weighed directly.

RESULTS AND COMMENT

General Observations.—The average results given in table 3 indicate that vitamin D was more effective with the high carbohydrate than with the high protein diet in promoting the growth of the rats and in improving their appetite, survival, calcium retention and calcification of bones. On the other hand, as is seen in the

TABLE 3.—*Average Results Obtained with Rats on Low Calcium Diets* *

	Rat Group I	Rat Group II	Rat Group III	Rat Group IV
	High Protein Diet		High Carbohydrate Diet	
	With D	Without D	With D	Without D
Initial weight, Gm.	100	105	100	105
Weight at 6 months, Gm.	245	246	288	237
Weight at 12 months, Gm.	265	256	313	262
Length at 6 months, cm.	22.1	22.5	23.2	21.4
Length at 12 months, cm.	22.4	22.8	23.9	22.8
Surface area at 6 months,† sq. cm.	340	341	375	334
Surface area at 12 months, sq. cm.	357	351	394	355
Mortality at 6 months, %	17	17	0	17
Mortality at 12 months, %	50	33	0	50
Diet, Gm. per Kg. per 24 hours	41.9	41.5	47.0	44.0
Diet, calories per Kg. per 24 hours	172	170	194	180
Diet, calories per sq. meter per 24 hours ‡	1,234	1,228	1,454	1,297
Calcium retained, mg. per Kg. per 24 hours	3.76 ± 0.20	4.69 ± 0.23	6.60 ± 0.22	4.89 ± 0.28
Calcium retained, % of intake	50.3 ± 3.1	61.2 ± 2.5	75.7 ± 2.9	59.6 ± 3.4
Calcium retained per rat per 24 hours	0.923 ± 0.058	1.14 ± 0.056	1.70 ± 0.072	1.15 ± 0.084
Calcium in bone, %	14.9	14.8	16.0	14.8
Calcium in incisors, %	26.2	24.7	26.1	22.5
Calcium in molars, %	30.0	29.7	29.3	28.4
Ash in bone, %	57.7	51.8	55.9	53.4
Ash in incisors, %	75.2	70.3	73.8	66.3
Ash in molars, %	76.0	75.3	74.7	74.8
Calcium in ash of bone, %	25.8	28.6	28.6	27.7
Calcium in ash of incisors, %	34.8	35.2	35.4	33.9
Calcium in ash of molars, %	39.5	39.5	39.2	37.9

* The first month, required for the depletion of vitamin D from the body stores of the rats, is omitted.
† The surface areas of the rats were calculated from a modified Meehs-Rubner formula (Lee and Clark [14]), $S = 12.54 \times Wt.^{0.40}$.
‡ The values for calories per square meter of body surface were based on the average daily food consumption of each group during each week and the average body weight of the rats of each group at the end of the corresponding week.

calcium analyses of the incisor teeth and in the roentgen and histologic evidence described in a paper that is to follow this,[5] vitamin D was effective with both diets in assisting the calcification of the incisors and in preserving the normal structure of both bones and incisor teeth.

The diets of the present investigation, if one excepts the rather high acid-forming property of the high protein diet, the low calcium content and the high phosphorus/calcium ratio, were favorable for the retention of calcium. A moderate amount of fat in the diet, as in the present instance, has been found to be more conducive to absorption of calcium [6] and to healing of rickets [7] than a large amount,

5. Boyle, P. E., and Wesson, L. G.: Influence of Vitamin D on the Structure of the Teeth and Bones of Rats on Low Calcium Diets, this issue, p. 243.
6. Boyd, O. F.; Crum, C. L., and Lyman, J. F.: J. Biol. Chem. **95**:29, 1932.
7. Knudsen, A., and Floody, R. J.: J. Nutrition **20**:317, 1940.

which decreases absorption of calcium.[8] Moreover, dextrin has been shown by Bergeim[9] to be more favorable for absorption of calcium than the other carbohydrates that he tested except lactose. Despite these favorable influences of the diets, it is difficult to understand, especially with regard to the groups not receiving supplemental vitamin D, the uniformly positive calcium balances and gradual increases of body weight that were found for each group of rats. If these are ascribed to the small amount of vitamin D contained in the butter fat of the diets, the question arises as to why this amount of D should favor the rats maintained on the high protein diet without the supplemental D (group II), whereas an increased amount of D (group I) is ineffective in increasing the retention of calcium or in improving the rate of growth. The results would seem to be analogous to the findings of Schneider and Steenbock[10] by which it was shown that vitamin D is growth inhibitory in phosphorus-deficient rats, except for the fact that the same amount of supplemental D was found to be favorable to growth in the corresponding high carbohydrate group of rats.

As already intimated, the positive calcium balance, the increase in weight and the survival time on both of the diets, with and without added vitamin D, was most surprising. The older than usual starting age of the animals is perhaps one factor in addition to the favorable features of the diets already mentioned. Rottenstein,[11] however, obtained positive calcium balances and weight increases using 30 day old rats on a diet containing 100 mg. of calcium and 190 mg. of phosphorus per hundred grams of diet. On the other hand, Boelter and Greenberg[12] found that cessation of growth appeared after as short a time as seven weeks with 90 Gm. rats on a diet containing 20 mg. of calcium per hundred grams of diet. These rats were amply supplied with cod liver oil and received 25 per cent casein and 25 per cent fat in their diet. In the present investigation the cessation of growth appeared only at the end of ten months in the groups fed the high protein diet. This discrepancy may be due partly to the fact that the rats of Boelter and Greenberg were fed a diet that contained a high percentage of fat, which reduces the absorption of calcium from the intestinal tract.

The body proportions were approximately the same in all of the groups if one may judge from the nutritive index, $N = \frac{Wt.^{1/3}}{L}$ of Cowgill and Drabkin,[13] which varied only from 0.278 to 0.289 for all of the groups at the end of the six month and twelve month periods. Furthermore, the surface areas of the rats at six and twelve months when calculated by the formula of Lee and Clark,[14] which uses this nutritive index, were nearly the same as those obtained by the use of their modified Meehs-Rubner formula for rats of normal proportions.[14] No symptoms of tetany were observable at any time in any of the groups.

The low calcium values for the leg bones of these animals[15] are comparable to the values found by others for the leg bones of rats on low calcium diets. For example, Brooke, Smith and Smith[16] found that adult rats on a diet containing

8. French, C. E.: J. Nutrition **23**:375, 1942.
9. Bergeim, O.: J. Biol. Chem. **70**:35, 1926.
10. Schneider, H., and Steenbock, H.: J. Biol. Chem. **128**:15, 1939.
11. Rottenstein, K. V.: Biochem. J. **32**:128, 1938.
12. Boelter, M. D. D., and Greenberg, D. M.: J. Nutrition **21**:61, 1941.
13. Cowgill, G. R., and Drabkin, D. L.: Am. J. Physiol. **81**:36, 1927.
14. Lee, M. O., and Clark, E.: Am. J. Physiol. **89**:24, 1929.
15. Dried leg bones of normal adult rats after extraction with alcohol and ether contain 23.4 to 24.1 per cent calcium (Kramer, B., and Shear, M. J.: J. Biol. Chem. **79**:147, 1928).
16. Brooke, R. O.; Smith, A. H., and Smith, P. K.: J. Biol. Chem. **104**:141, 1934.

ample vitamin D but low in salts gave in the fat-free, dry, leg bones in ninety days 17.6 per cent calcium, compared with 30.4 per cent calcium on a favorable intake of salts.

The incisor teeth of the rats were relatively well calcified on both of the diets notwithstanding the low intake of calcium. Karshan and Rosebury [17] after feeding young rats a rachitogenic low calcium diet found 23.5 per cent calcium in the incisors despite the low calcium content of the bones. When they supplied ample calcium, the value obtained was 25.8 per cent.

The possibility that the added fluorine content of the diet (0.0009 per cent fluorine; the salt mixture of Osborne and Mendel [2a] and that of Hawk and Oser [18] both give 0.0010 per cent added fluorine in the ration) affected the calcification of the teeth and bones in the presence of the low percentage of calcium is an unknown factor. Hauck, Steenbock and Parsons [19] found that with a diet containing 0.1 per cent calcium an added fluorine content of 0.068 per cent lowered the bone ash from 38.9 to 36.1 per cent and the incisor ash from 64.8 to 57.2 per cent. According to Schour and Smith,[20] 0.011 per cent fluorine causes a disturbance in the calcification of the incisors, indicated by chalky white enamel, but lower levels, even though producing severe dental fluorosis, do not measurably alter the calcium and phosphorus metabolism.

High Protein Diet (Groups I and II).—Marked retardation of growth was found in the rats on this diet, but the retardation was no greater in general than that in the high carbohydrate group without supplemental D (group IV). Hamilton [21] fed diets containing up to 54 per cent protein to rats at the starting age of 4 weeks and found 16 to 30 per cent protein diets of equal growth-producing value. Since young rats are more susceptible to adverse diets than the older ones used in the present work, it is possible that the 38 per cent protein of the present diet was not unfavorable so far as their growth and activity were concerned.

The most striking observation with regard to the high protein diet was the complete lack of the beneficial effects of vitamin D on retention of calcium, the calcium content of the bones and the growth and survival time of the rats. For this phenomenon we have no explanation, nor can we account for the fact that the retention of calcium (per kilogram of body weight, per cent of intake and per rat) of the rats of group I (with D) was even somewhat poorer than that of group II (without D).

That vitamin D was able to function in the rats on the high protein diet was shown by the greater calcium content of the incisors and the more nearly normal structure of the bones and of the teeth of the rats of group I as compared with those of group II. The molar teeth likewise reflected the action of the supplemental vitamin D to a certain extent. The possibility that such action can occur may be explained by the findings of Hoffman and Schour [22] that a continuous formation of secondary dentin takes place in rat molars. This secondary dentin, if less calcified in the case of the rats of group II because of the deficiency of vitamin D, would reduce the calcium content of the molars as compared with those of group I in which vitamin D was not deficient.

17. Karshan, M., and Rosebury, T.: J. Dent. Research **13**:305, 1933.

18. Hawk, P. B., and Oser, B. L.: Science **74**:369, 1931.

19. Hauck, H. M.; Steenbock, H., and Parsons, H. T.: Am. J. Physiol. **103**:480, 1933.

20. Schour, I., and Smith, M. C.: Experimental Dental Fluorosis, in Fluorine and Dental Health, Publication 19, American Association for the Advancement of Science, 1942, p. 32.

21. Hamilton, T. S.: J. Nutrition **17**:565, 1939.

22. Hoffman, M. M., and Schour, I.: Anat. Rec. **78**:233, 1940.

High Carbohydrate Diet (Groups III and IV).—The results obtained with the rats on this diet, which contained protein still within the optimal range, serve as controls for comparison with those obtained with the rats on the high protein diet. A marked beneficial effect of vitamin D on all of the factors studied was found with the rats on the high carbohydrate diet. In the group in which supplemental D was used (group III) there was no visible indication of any abnormality except for the slow rate of growth. As the increase in weight was still taking place at the end of twelve months, there was every indication that continuation of the experimental period would eventually result in a normal adult size for the rats of this group. This increase in weight during the twelve months was 213 Gm., or 35 per cent more than that of the group on the same diet but without supplemental vitamin D. The amount of calcium retained was likewise 35 per cent greater.

CONCLUSIONS

Vitamin D has a markedly favorable influence on the growth and survival time of rats maintained on a high carbohydrate–low calcium diet, as well as an appreciable effect in improving the appetite, the retention of calcium and the calcium content of the bones. On a high protein–low calcium diet none of these factors is improved by supplemental D. The calcification of the teeth and the structure of the bones and of the teeth are favored by vitamin D with both diets.

These findings suggest that the protein content of the diet of rats should be kept at a moderate level when studies of the retention of calcium and the effectiveness of vitamin D are made.

SUMMARY

Two low calcium diets, one high in protein and the other in carbohydrate but otherwise substantially alike, were fed to rats over a deficiency period that lasted twelve months. Both of these diets were used with and without supplemental vitamin D.

During this time vitamin D had a pronounced effect in increasing the growth and survival of the rats on the high carbohydrate diet, as well as an appreciable effect in improving the appetite, the retention of calcium and the calcium content of the bones, but had no effect in improving these factors in the rats on the high protein diet.

On the other hand, the degree of calcification of the teeth and the structure of both the bones and the teeth were improved on both diets by the supplemental vitamin D.

INFLUENCE OF VITAMIN D ON THE STRUCTURE OF THE TEETH AND OF THE BONES OF RATS ON LOW CALCIUM DIETS

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The purpose of this paper is to present evidence of a direct effect of vitamin D on the structure of the teeth and the bones of rats maintained for one year on diets low in calcium. The details of the experimental procedure, including the selection and the care of the animals, the composition of the diets and the chemical procedures, are given in a preceding publication.[1]

Two diets were used: one high in protein (groups I and II) and one high in carbohydrate (groups III and IV). The phosphorus/calcium ratio of both diets was approximately 16 to 1. Half of the animals on each diet were given

TABLE 1.—*Data Showing General Effects of Vitamin D in Groups of Rats on High Protein and High Carbohydrate, Low Calcium Diets* *

Group †	Average Initial Weight, Gm.	Weight at 12 Mo., Gm.	Surface Area at 12 Mo., Sq. Cm.	Mortality at 12 Mo., %	Calcium Retention, % of Intake	Calcium in Bone, %
I, high protein diet with vitamin D ‡.....	100	265	357	50	50.3 ± 3.1	14.9
II, high protein diet without vitamin D....	105	256	351	33	61.2 ± 2.5	14.8
III, high carbohydrate diet with vitamin D ‡.	100	313	394	0	75.7 ± 2.9	16.0
IV, high carbohydrate diet without vitamin D	105	262	355	50	59.6 ± 3.4	14.8

* The P/Ca ratio of all diets was approximately 16 to 1.
† Each group consisted of 6 animals.
‡ The vitamin D amounted to 272 U. S. P. units per hundred grams of diet.

272 U. S. P. units of vitamin D as viosterol per hundred grams of diet (groups I and III). From the data presented in table 1 it appears that vitamin D was effective only in the animals on the high carbohydrate diet (group III). In this group of animals there was an increased rate of growth, a lessened mortality rate, a higher percentage of retention of calcium and an increased calcium content of the bones of the femurs. Animals on the high protein diet supplemented with vitamin D (group I) showed no improvement in these respects over the animals on either the high protein (group II) or the high carbohydrate (group IV) diet without vitamin D (table 1).[2] Histologic examination of the teeth, however, indicated that vitamin D had a marked effect with both the high protein and the high carbohydrate diet in improving calcification, as indicated by the shape of the teeth, the character of the calcification of the enamel and the dentin and the width of the predentin. Chemical analysis of the teeth also showed an appreciable effect of vitamin D in improving the percentage of calcium and of ash in the incisor

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1. Wesson, L. G., and Boyle, P. E.: Arch. Path., to be published.

2. As noted in the previous publication,[1] the diet of all animals contained 4 per cent butter fat, which furnished a small amount of vitamin D.

teeth (table 2). The molar teeth showed a slightly greater calcium content but essentially the same percentage of ash. No great change was to be expected in these teeth, since the greater part of them was formed and calcified before the start of the experiment. The secondary dentin which continues to form throughout the life of the animals was histologically less well calcified in the animals not receiving vitamin D.

The ash in bone was greater in animals receiving vitamin D. Histologically it was evident that vitamin D also prevented the active resorption of trabecular and cortical bone, "osteitis fibrosa," found in animals on the high protein and the high carbohydrate diet not supplemented with vitamin D.

Ross [3] described the effect of viosterol in inducing the calcification of trichinal cysts in vitro, and Kraemer and co-workers,[4] the influence of viosterol on bone-forming cells. Irving [5] reported healing of low phosphorus rickets (Ca/P ratio = 4.8) produced by a single dose of vitamin D (9.2 U. S. P. units) as measured by improved calcification of the incisor teeth of rats. Gaunt and Irving [6] demonstrated that diets with a low calcium/phosphorus ratio (Ca/P = 0.5) interfere with calcification of the teeth of rats. Boelter and Greenberg [7] described briefly the histologic changes in the bones of growing rats in severe calcium deficiency. They noted that porosis of the tibial diaphysis was pronounced and that the compact lamellation present in normal bone was not present in the bones from the calcium-deficient animals except for very limited, thin periosteal and endosteal shells. They further pointed out changes in the bone trabeculae and in the appearance and the number of osteocytes and osteoclasts which somewhat resembled conditions produced by certain stages of dosage with parathyroid hormone. These changes were similar to those to be reported in our animals not receiving viosterol.

TABLE 2.—*Data Showing Effects of Vitamin D on the Bones and the Teeth of Rats on High Protein and High Carbohydrate, Low Calcium Diets*

Group	Calcium in Bone, %	Calcium in Incisors, %	Calcium in Molars, %	Ash in Bone, %	Ash in Incisors, %	Ash in Molars, %
I, with vitamin D	14.9	26.2	30.0	57.7	75.2	76.0
II, without vitamin D	14.8	24.7	29.7	51.8	70.3	75.3
III, with vitamin D	16.0	26.1	29.3	55.9	73.8	74.7
IV, without vitamin D	14.8	22.5	28.4	53.4	66.3	74.8

METHOD OF PREPARATION OF THE SPECIMENS FOR HISTOLOGIC EXAMINATION

The heads of the animals from each of the four groups were split in a midsagittal plane, roentgenograms made and the specimens fixed in 4 per cent neutral solution of formaldehyde. After being decalcified with 5 per cent nitric acid and embedded in celloidin, the upper incisor teeth were cut in longitudinal sections, and these were stained with hematoxylin and eosin. One half of the mandible of each animal, including the lower incisor and the three molar teeth, was cut in cross section at three levels. These levels were just anterior to the first molar tooth, between the first and second molar teeth, and approximately midway of the third molar tooth. Sections from the anterior face of each of the resulting blocks of tissue were cut without decalcification, ground, polished and mounted for examination. The remainder of the blocks was then decalcified and prepared for microscopic examination in the usual manner.

3. Ross, B. D.: Proc. Soc. Exper. Biol. & Med. **45**:531, 1940.
4. Kraemer, V. V.; Landtman, B., and Simola, P. E.: Acta physiol. Scandinav. **1**:285, 1940.
5. Irving, J. T.: Nature, London **147**:608, 1941.
6. Gaunt, W. E., and Irving, J. T.: J. Physiol. **89**:18, 1940.
7. Boelter, M. D. D., and Greenberg, D. M.: J. Nutrition **21**:61, 1941.

In this way, both undecalcified and decalcified sections of the teeth at different levels were made available for examination. The other half of the mandible was decalcified, and sections were cut in a sagittal plane.

A roentgenogram was made of the bones of one hindleg of each animal and the bones then preserved in 80 per cent alcohol. These bones were split, and one half was stained with silver nitrate by the method of Gömöri.[8] The other half was decalcified and prepared for histologic examination

OBSERVATIONS

Gross Changes.—The pigment of the incisor teeth was abnormal in distribution and intensity, particularly in those animals which received no vitamin D. In the upper incisor teeth the enamel, which is normally a deeper color than that of the corresponding lower teeth, was in some instances chalky white. In other animals blotches of yellow pigment were irregularly distributed through the enamel. The enamel of the corresponding lower incisor teeth was relatively less affected but in general it was more lightly and irregularly pigmented than normal. Gaunt and associates[9] described similar changes in the incisor teeth of rats on a low calcium diet.

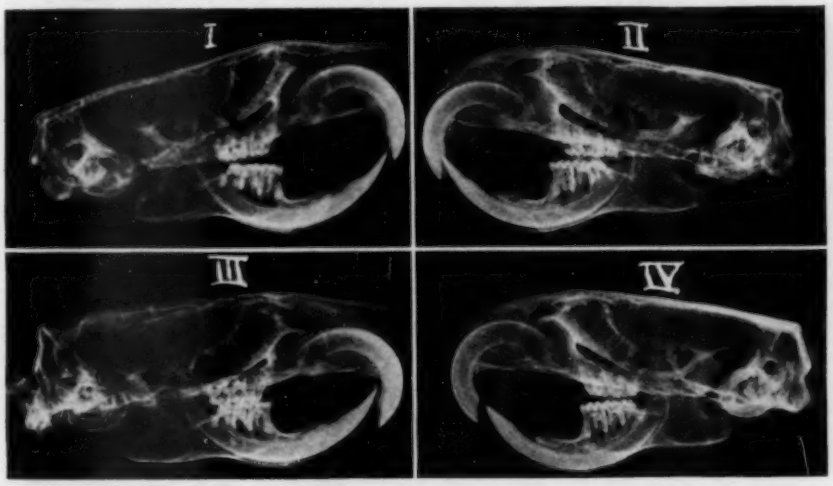

Fig. 1.—Roentgenograms of half skulls of rats from the four groups reported. Rats in groups I and III received supplemental vitamin D, whereas those in groups II and IV did not. The heavy cortical bone of the calvarium and the relatively coarse trabecular pattern of animals II and IV should be noted. The incisor teeth of animals I and III cast a relatively dense shadow and show smaller pulp chambers than do the teeth of the vitamin-deficient animals.

The pigment of the incisor teeth of the animals receiving vitamin D (groups I and III) appeared of normal or slightly less than normal intensity and was for the most part uniformly distributed. However, on examination with the dissecting microscope the enamel of the lower incisor teeth of these groups was found to exhibit fine cross striations of alternating dark and light bands of pigment. The pairs of lines averaged approximately 150 microns in width. The upper incisor teeth did not exhibit this banding. The lower incisor teeth of the animals in groups II and IV usually showed much coarser and less regular bands of pigment. Coarse banding of the teeth has been described in fluorosis by Smith and Lantz.[10] A fine

8. Gömöri, G.: Am. J. Path. **9**:253, 1933.

9. Gaunt, W. E.; Irving, J. T., and Thompson, W.: J. Hyg. **39**:91, 1939.

10. Smith, M. C., and Lantz, E. M.: Experimental Production of Mottled Enamel, Technical Bulletin 45, University of Arizona, 1933.

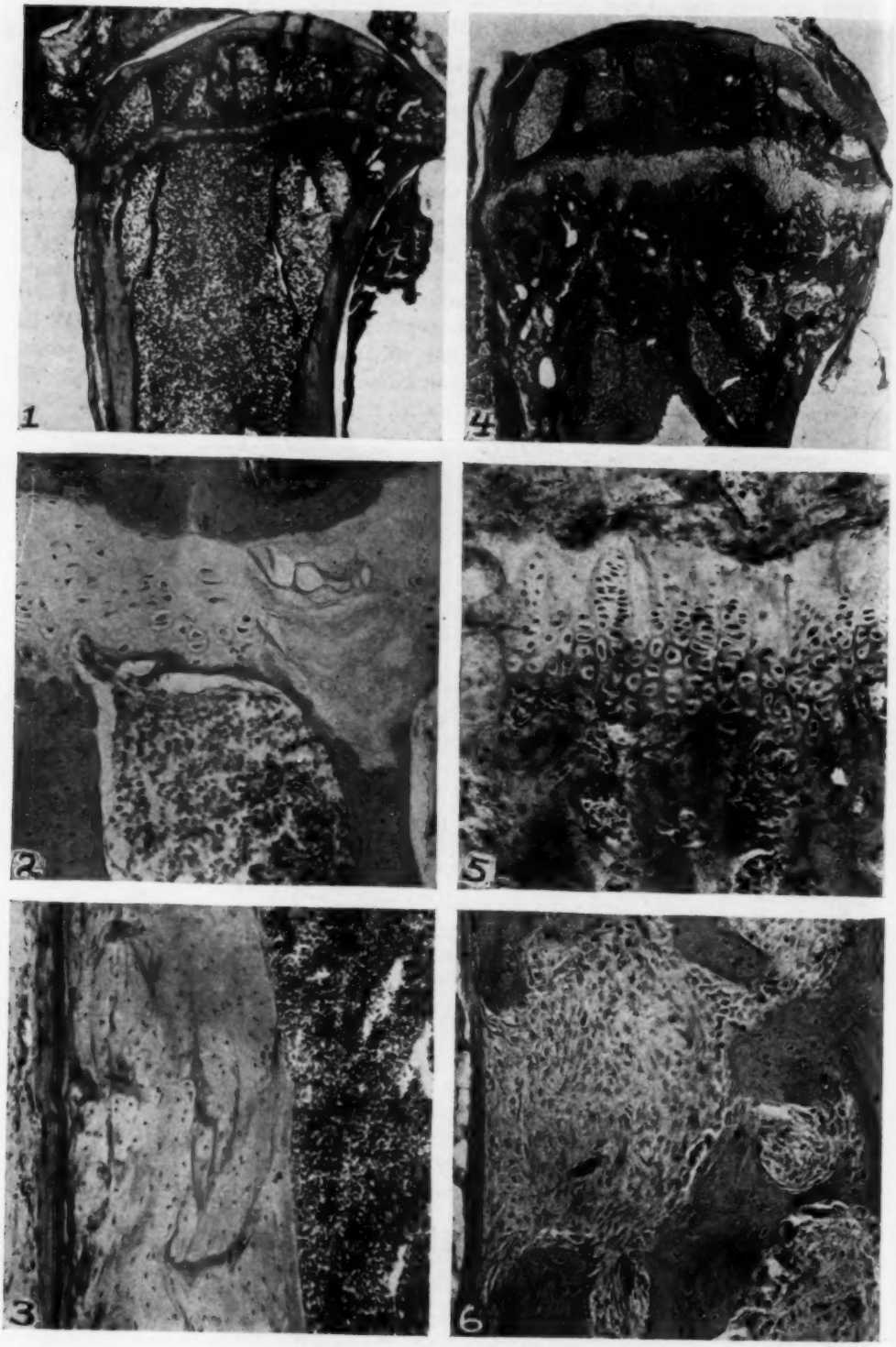

Figure 2

(See legend on opposite page)

banding, discernible only when the teeth are examined with a magnifying glass, has been found to occur in animals fed a diet containing as little as 7 parts per million of fluorine (Schour and Smith [11]).

Caries was not observed in the teeth of any of the animals.

Roentgen Findings.—The bones of animals of all the groups showed osteoporosis. In the bones from the rats receiving vitamin D (groups I and III) the cortex and the bony trabeculae appeared more delicate. The trabeculae were fewer, so that the pattern of the bony structure was more easily discernible. This was especially striking in the cortical bone outlining the tooth sockets and in the supporting trabeculae, but it was noted in other parts of the skull (fig. 1) and also in the femurs and the tibias.

A striking variation was the decidedly greater density of the teeth of the animals receiving vitamin D (groups I and III). The pulp chambers in the teeth of these animals were smaller, and there was less contrast between the shadows cast by the enamel and the dentin than in the roentgenograms of the animals not receiving vitamin D (fig. 1).

Histologic Changes in the Bones.—In the rats that received vitamin D (groups I and III) the cartilage of the epiphysial plate at the proximal end of the tibia was fairly uniform and relatively narrow. In the rats receiving no supplemental vitamin D (groups II and IV) the width of the epiphysial cartilage was generally greater and the cartilage cells were more numerous and larger than in groups I and II. Several animals showed fairly long tongues of cartilage cells projecting irregularly into the diaphysis. Resorption and new formation of both the trabecular bone and the cortical bone of the shaft, with presence of coarse fibrous tissue in the spaces thus formed, was a consistent and striking change in the animals not receiving vitamin D. Osteoclastic resorption was prominent in these areas. The appearance closely resembled that of "osteitis fibrosa" due to hyperparathyroidism (fig. 2). No prominent osteoid margins were observed on the bone trabeculae or on the cortical bone of the diaphysis of any of the animals.

Histologic Changes in the Teeth.—The incisor teeth of the animals that received no supplemental vitamin D showed evidence of interference with the normal deposition and calcification of enamel and dentin. The cross-sectional outline of the tooth was distorted with a marked bulge of the lingual side of the tooth, which is normally straight (fig. 3, *1* and *4*). This distortion of the tooth under stress of function may be compared to the bowing of the legs in rickets. The matrix of enamel tended to remain in sections after decalcification, in contrast to similarly

11. Schour, I., and Smith, M. C., in Fluorine and Dental Health, Publication 19, American Association for the Advancement of Science, 1942.

EXPLANATION OF FIGURE 2

The photomicrographs on the left are from tibias of animals receiving supplemental vitamin D; those on the right, from animals not receiving the supplement. *1* shows relatively few trabeculae and a narrow epiphysial cartilage in contrast with *4*. × 25.

2 and *5* show details of the epiphysial cartilage and bone trabeculae. The cartilage cells are more numerous and larger in *5*, and the formation of bone trabeculae is still taking place. Areas of acellular, degenerating cartilage are shown in both sections. × 146.

3 and *6* are areas from the diaphysis. In *3* dense cortical bone is covered on the left by periosteum and on the right endosteum and bone marrow. In *6* the cortical bone immediately under the periosteum has been resorbed, leaving a fibrous-appearing soft tissue. Osteoblasts and osteoclasts are prominent about the remaining bony structures. × 146.

treated sections of the teeth of the animals receiving vitamin D. Areas of disruption of the enamel organ were found, with islands of enamel formation among the misplaced enamel-forming cells (fig. 3, *5*). The enamel organ farther incisally became atrophic, the ameloblasts appearing flattened in a region where they should

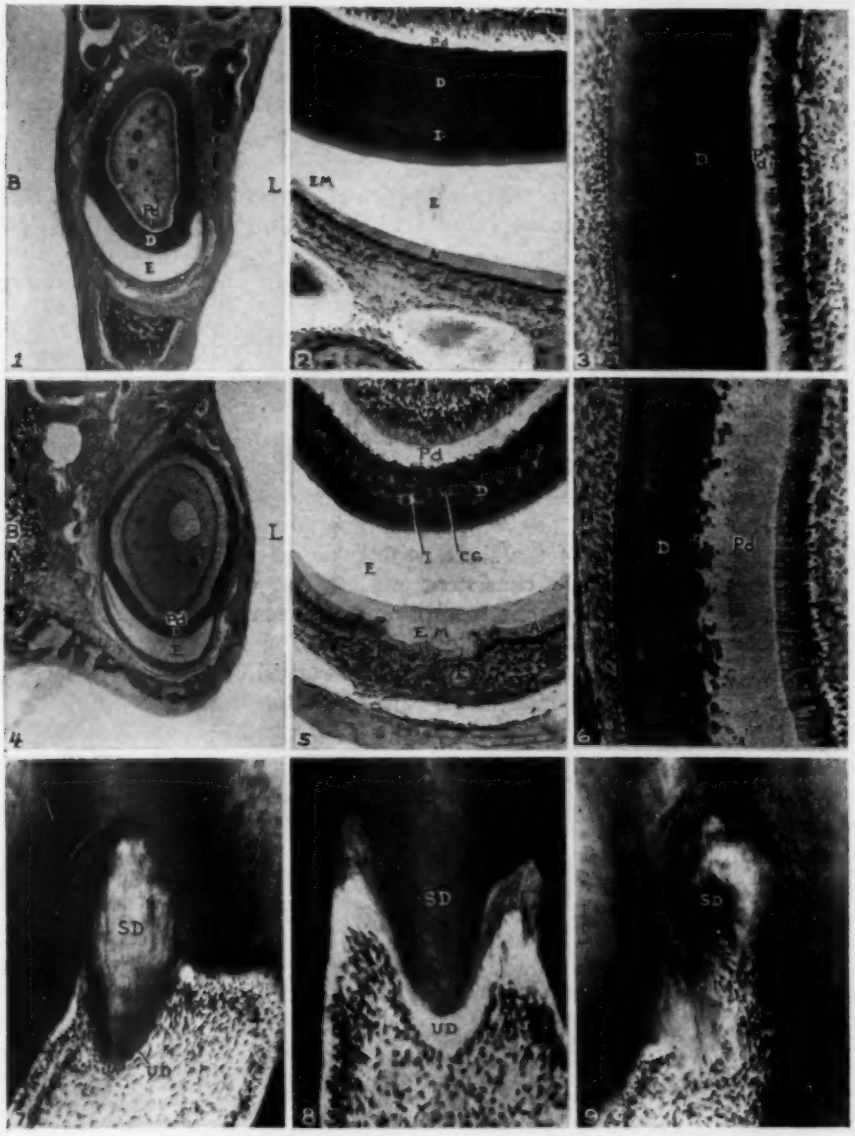

Figure 3

(See legend on opposite page)

normally retain a columnar form. This atrophy of the ameloblasts presumably was responsible for the disturbance of the pigmentation of the enamel.

The dentin of the incisor teeth of the rats receiving supplemental vitamin D appeared almost normal. Small, lightly staining interglobular areas were found under the enamel-covered surface (fig. 3, *2*) and to a lesser extent under the

cementum-covered surface (fig. 3, *3*). The dentin-predentin border was relatively even, and the predentin was of normal width. In contrast, the dentin of the animals not receiving supplemental vitamin D was strikingly abnormal. Only the peripheral zone appeared uniformly calcified. The remainder of the dentin showed large, deeply stained calcoglobules, between which lightly stained areas appeared. The dentin-predentin border was irregular, and a number of small calcoglobules were found in this region. The predentin was abnormally wide, in some instances measuring as much as 100 microns (fig. 3, *6*).

In unstained ground sections the relatively uniform calcification of the dentin and the clear, narrow predentin of the teeth of the animals receiving vitamin D were in marked contrast to the granularity of the dentin and the wide predentin of the teeth of the vitamin D–deficient animals. When the ground sections were subsequently treated with 1 per cent silver nitrate and exposed to sunlight, the dentin became black and the predentin remained unstained.

Measurement of the rate of formation of dentin by the injection of alizarin red S was not undertaken, but the widths of the dentin at similar cross-sectional levels of the teeth indicated that the rate of formation was reduced in the animals not receiving supplemental vitamin D.

The greater part of the molar teeth was formed before the beginning of the experiment, so that one would expect to find the effects of the dietary regimen only in the secondary dentin and cementum (Schour and Hoffman[12]). Secondary dentin was deposited under the cusps of the molar teeth to compensate for the wearing away of the biting surface. In the rats receiving supplemental vitamin D the secondary dentin appeared well formed and calcified (fig. 3, *7*). Animals receiving no supplemental vitamin D showed secondary dentin with a wide uncalcified border (fig. 3, *8*). In some instances the secondary dentin was scanty and irregularly formed, and included atrophic odontoblasts (fig. 3, *9*). This is similar to the findings of Mellanby[13] concerning the formation of secondary dentin in the teeth of rachitic dogs.

12. Schour, I., and Hoffman, M. M.: J. Dent. Research **18**:91, 1939.
13. Mellanby, M.: Proc. Roy. Soc. Med. (Sect. Odontol.) **16**:74, 1923.

Explanation of Figure 3

1 is a photomicrograph of a cross section of an incisor tooth of a rat receiving vitamin D. The buccal side (*B*) is curved, and the lingual side (*L*) is nearly straight. The enamel space (*E*) left by dissolution of the enamel on decalcification is normal in outline. The dentin (*D*) is uniformly calcified and the predentin (*Pd*) is narrow. × 12.5.

2 and *3* are detailed views of enamel-covered and cementum-covered (buccal) areas of the same tooth. In *2* a slight amount of enamel matrix (*EM*) is present indicating that the enamel was not completely removed by treatment with acid and, therefore, that it was slightly hypocalcified. The small interglobular areas (*I*) indicate that the dentin (*D*) was also slightly hypocalcified. × 73.

4 is a cross section of an incisor tooth of a rat not receiving vitamin D. × 12.5. The buccal (*B*) and lingual (*L*) sides of the tooth are bowed in the vitamin D–deficient animal. *5* and *6* are detailed views for comparison with *2* and *3*. The dentin (*D*) in *5* and *6* is narrow and contains prominent calcoglobules (*CG*) and interglobular spaces (*I*). The predentin (*Pd*) is very wide. The ameloblasts (*A*) in *5* are irregularly arranged and enamel (*E*) has been formed within the enamel organ. An abnormal amount of enamel matrix (*EM*) remains after decalcification. × 73.

7 shows an area of a molar tooth of a rat receiving vitamin D. There is a narrow seam of uncalcified dentin (*UD*) about the border of the secondary dentin (*SD*) which has filled in a horn of the pulp chamber. *8* and *9* show similar areas from teeth of animals not receiving vitamin D. The uncalcified dentin seam is much wider, and in *9* the secondary dentin is irregularly formed. × 73.

COMMENT

The finding that animals receiving supplemental vitamin D showed histologically better bone structure than those not receiving the supplement is similar to observations reported by Nicolaysen and Jansen.[14] These investigators attempted to determine whether vitamin D has or has not a direct action on bone formation. Their experiments were designed "to transfer identical amounts of calcium and phosphorus into the blood stream of vitamin D–deficient rats and of rats given a liberal supply of vitamin D, and to study the anatomic and chemical effect on the bones." They used the Steenbock-Black rachitogenic diet no. 2965 during a thirty day preparatory period, which was followed by the experimental period proper. They concluded that the density of the calcification of the bones as measured by chemical analysis appeared to be governed solely by the amounts of calcium and phosphorus brought to the blood. The histologic examination, however, indicated that vitamin D either directly or indirectly may influence the bony structure. This assumption was based largely on appearances in 7 month old rats kept for one hundred and thirty-five days on the Steenbock-Black ration with 3 per cent calcium phosphate substituted for the 3 per cent calcium carbonate. During the following thirty-five days one group of animals was continued on the same diet and another group was given 35 U. S. P. units of vitamin D daily. Histologically, the most striking feature in the bones of animals of the first group was the density of the substantia spongiosa. In the second group, with vitamin D, the trabeculae were considerably fewer and the bone marrow was cellular, whereas in the first group it was fibrocellular. The dense network of trabeculae in the substantia spongiosa was characteristic also of animals kept on other, slightly modified diets without vitamin D and was in marked contrast to "few but strong trabeculae separated by cellular marrow" in those given vitamin D. These workers did not report examinations of the teeth of their animals.

The fact that the bone structure was more nearly normal in our animals on a high protein diet (group I) in spite of the fact that there were no beneficial effects of vitamin D on the retention of calcium, on the calcium content of the bones or on the growth and the survival time of the rats is additional evidence that vitamin D may promote normal calcification even though it is ineffective in improving the retention of calcium.

Gaunt and Irving [6] found that with an inadequate intake of calcium and phosphorus the calcification of the teeth was more disturbed when the calcium intake was relatively low and the phosphorus intake high, whereas the calcification of bone was poorer with diets having high calcium/phosphorus ratios. They reported the results of chemical analysis of the bone as well as of the teeth, but apparently studied only the teeth histologically. They found that the histologic picture of wide predentin and globular calcification of the dentin correlated well with the dentin ash content of the incisor teeth. The fact that bone trabeculae do not show osteoid margins on low calcium diets with low calcium/phosphorus ratios helps to explain the relatively high ash content of the bone in such animals. Since these diets cause the formation of bone trabeculae without osteoid margins, whereas rachitogenic diets with high calcium/phosphorus ratios cause the formation of bone trabeculae with wide osteoid margins, it is not surprising that there is a relatively greater reduction in percentage of bone ash in the latter condition. The formation of a wide predentin margin in the incisor teeth while the bone trabeculae show no comparable osteoid margins in animals on low calcium diets without adequate vitamin D remains unexplained. The predentin was abnormally wide in

14. Nicolaysen, R., and Jansen, J.: Acta pædiat. **23**:405, 1939.

the teeth of our animals on low calcium, low vitamin D diets only in the basal and middle parts of the tooth. Toward the incisal part of the tooth the predentin became narrower and then disappeared as the formation of osteodentin closing off the pulp chamber occurred. Thus the dentin formed by odontoblasts which had recently differentiated and come into function showed a wide uncalcified border, while the dentin formed by odontoblasts which were completing their life cycle was calcified almost if not quite to the cell border. In a previous report [15] on the production of low calcium rickets in the guinea pig, it was noted that only the younger, rapidly growing animals showed bone trabeculae with wide osteoid margins. In older animals arrest of growth occurred and presumably was responsible for the disappearance of this histologic sign of rickets. The teeth of all animals, however, showed a wide uncalcified border of dentin (dentinoid or predentin) similar to that described for the teeth of rats. Toward the incisal end of the tooth the odontoblasts formed dentin which was calcified to the ends of the cells. Thus in both the rat and the guinea pig senescent odontoblasts formed calcified tissues without an intervening uncalcified border and were in this respect similar to osteoblasts adjacent to calcified bone trabeculae. Odontoblasts which had recently differentiated from the embryonic-like tissues at the formative end maintained a greatly widened zone of uncalcified dentin between the ends of the cells and the calcified dentin. They resembled the osteoblasts and the bone trabeculae which are characteristic of low phosphorus rickets but which are found in association with low calcium rickets only in the rapidly growing bones of young animals.

In adult rats the incisor teeth continue to form and to erupt, in contrast to bone growth, which becomes stationary. These teeth are constantly renewed by the formation of new structures at the basal ends to replace the tooth substance worn away at the incisal ends. Since the process takes about fifty days, the teeth were renewed several times during the experimental period. There is no normal process of resorption and replacement of dental structures comparable to the physiologic process of resorption and reformation of bone. The teeth accordingly registered an uncomplicated picture of tooth matrix formation and calcification during the experimental period. In contrast, bone matrix formation and calcification are complicated by the physiologic replacement of primary trabeculae by secondary trabeculae, and of these by cortical bone, the whole being subject to rearrangement to withstand functional stress as well as serving as a reservoir of mineral salts.

The teeth of rats on our low calcium diets supplemented with vitamin D indicated that the formation and the calcification of dentin were practically normal in spite of adverse conditions. The histologic appearance in the bones indicated that both the trabeculae and the cortical bone were in a state of relative equilibrium, although the absolute amount of bone was less than normal.

The histologic appearance of the teeth of rats on diets not supplemented with vitamin D suggested that the formation of the dentin matrix was essentially normal but that the calcification was markedly subnormal. The distortion of the tooth, the irregular globular deposits in the dentin and the extremely wide predentin were all striking manifestations of imperfect calcification.

In bone the evidence of imperfect calcification was indirect rather than direct. The increased number of trabeculae suggested a compensatory formation of excess bone, the individual components of which were inadequate to withstand functional stress. The active resorption of these trabeculae and also of the cortical bone may

15. Howe, P. R.; Wesson, L. G.; Boyle, P. E., and Wolbach, S. B.: Proc. Soc. Exper. Biol. & Med. **45**:298, 1940.

possibly be explained by the fact that the imperfect structure excited an osteoclastic action to effect its removal, while new bone was continually being formed in an attempt to maintain function. The situation suggests comparison with the hyperactive formation and the subsequent destruction of imperfect red blood corpuscles which occur in pernicious anemia. One is tempted to postulate a function of vitamin D in promoting the normal calcification of bones and of teeth, even under extremely adverse conditions, in a manner somewhat comparable to that of the "liver factor" in promoting the normal maturation of the erythrocytes in pernicious anemia.

SUMMARY

Vitamin D was effective with a high carbohydrate–low calcium diet in markedly promoting the growth of rats and in appreciably improving their appetite, their retention of calcium and the degree of calcification of their bones. Vitamin D was ineffective in these respects with a high protein–low calcium diet.

Vitamin D was effective in improving the structure of bones and teeth with the high protein–low calcium and the high carbohydrate–low calcium diets. The teeth of animals on both vitamin-deficient diets were characterized by wide, uncalcified predentin and dentin showing globular calcification, whereas the teeth of animals on the diets supplemented with vitamin D were histologically almost normal. The bone trabeculae of rats on either of the diets, with or without supplemental vitamin D, showed little or no osteoid formation. The bone shafts and bony trabeculae of animals on the diets supplemented with vitamin D were atrophic but otherwise almost normal. The bony structures of animals on the vitamin D–deficient diets showed active resorption and reformation with production of fibrous marrow spaces which simulated the "osteitis fibrosa" of hyperparathyroidism. It is suggested that this process may be explained on the basis of a formation of functionally inadequate bone on low calcium, vitamin D–deficient diets.

INCOMPLETE RUPTURE OF THE AORTA, NOT FOLLOWED BY DISSECTING ANEURYSM

REPORT OF TWO CASES

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The degenerative and necrotic changes of medionecrosis cystica idiopathica aortae from the beginning until to the final stages—dissecting aneurysm and so-called spontaneous rupture—have been frequently observed and described. Recently in an excellent paper Sailer [1] reviewed the development of the knowledge of this interesting, although rare, pathologic entity. Rupture of the aorta, a frequent accident in this disease, may be complete, with a fatal hemorrhage following, or incomplete. Nicholls [2] in 1728 by experimental overdistention produced bursting of the internal coats of the pulmonary artery with outpouching of the external ones. It was generally accepted then that when incomplete rupture of a vessel had occurred the blood forced a way between the coats of the vessel so that dissecting aneurysm developed. But several instances of dissecting aneurysm without intimal tear led Kruckenberg [2] in 1920 to the assumption that the blood of such an aneurysm proceeded from hemorrhages of the vasa vasorum, the intimal tear not being the entrance but the outlet of the aneurysmal blood. This view has been confirmed several times since—for instance, by Kaufmann,[3] who stated that rupture of the inner coats of the aorta may heal without dissection, leaving a furrow or a gaping cleft. In a case observed by him in a postmortem examination ten weeks after the rupture had occurred, the media appeared in the ground of the cleft, only partially covered by thrombi; there was but little dissecting. Eskelund [4] reported an instance of an aneurysm that developed after the healing of a spontaneous rupture at the root of the aorta, when the inelastic scar tissue which formed on the place of rupture yielded gradually to the pressure of the blood.

REPORT OF CASES

In the following pages 2 cases of incomplete rupture will be described. One of the ruptures was still in the healing stage at necropsy; the other was nearly scarred, but a second fresh rupture had occurred that was complete and fatal.

CASE 1.—A man 46 years old, who used to drink and smoke without excess, had felt some trouble from the heart during several years. Beginning in February 1942, edema of both legs, ascites and other symptoms of heart failure developed. In May he entered the hospital, and a month later he left greatly improved. The hospital records state that the heart was considerably enlarged, especially the left ventricle, and also the ascending aorta. There was slight insufficiency of the aortic valves. The blood pressure was 150 mm. of mercury maximum and 45 mm. minimum; Kahn's reaction was negative. The patient was admitted again to the hospital September 4, much worse than the first time, but during the following week, with heart-supporting therapy, he improved to a certain extent. He died suddenly September 27.

From the Institute of Pathological Anatomy, University San Francisco Xavier Medical School.

1. Sailer, S.: Arch. Path. **33**:704, 1942.
2. Cited by Sailer.[1]
3. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie für Studierende und Ärzte, ed. 9, Berlin, W. de Gruyter & Co., 1931.
4. Eskelund, V.: Ugesk. f. læger **103**:227, 1941; abstracted, J. A. M. A. **117**:814, 1941.

Necropsy.—The examination was begun twenty-nine hours post mortem. As the immediate cause of death a pulmonary embolus was found, apparently from the iliac veins, which, as well as the femoral veins, were thrombosed.

The pericardium contained about 300 cc. of a clear serous liquid. The heart was enlarged, measuring about 15 cm. in transverse as well as in longitudinal diameter; the apex was formed by the left ventricle only. The coronary arteries were normal. The myocardium was firm;

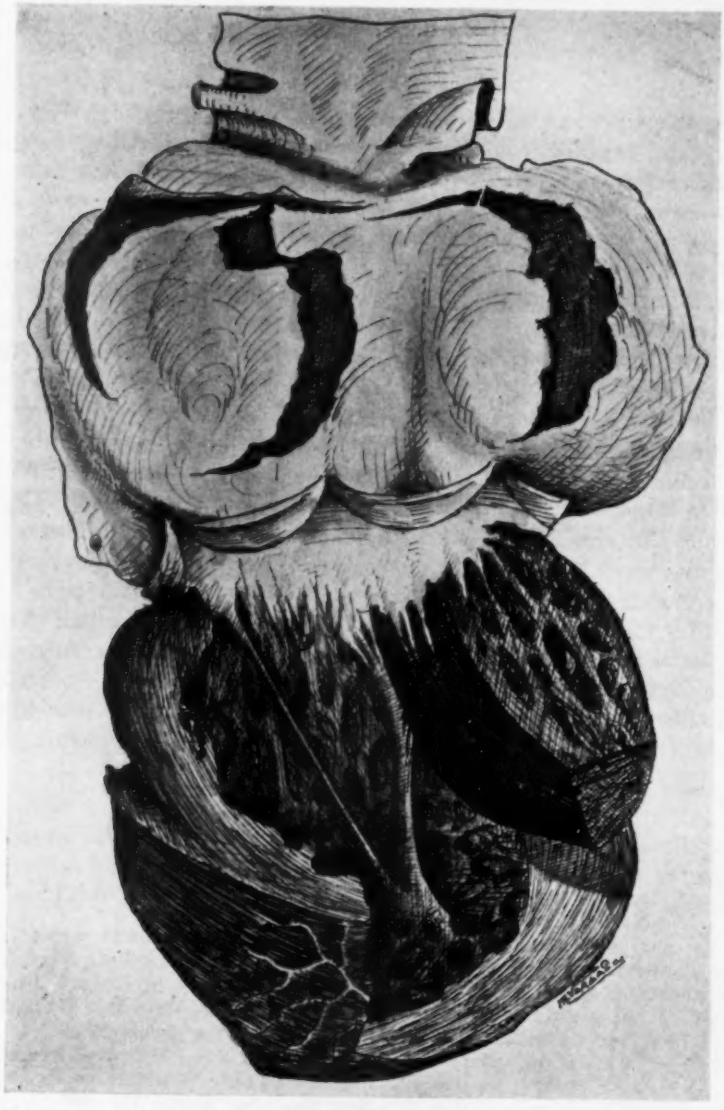

Fig. 1.—Drawing of the heart and the ascending aorta in case 1 showing the incomplete rupture of the aorta and the hypertrophy of the heart.

its thickness was on the average 15 mm. on the left and 6 mm. on the right side. All the trabecular and papillary muscles appeared hypertrophied; both ventricular cavities were dilated. All the valvular cusps were normal.

The aorta measured in its origin 10 cm. in circumference; 2 cm. above the origin it measured 16 cm.; toward the arch its caliber diminished abruptly; immediately before the origin of the brachiocephalic artery it was but 8 cm. around. The dilated ascending aorta was not covered completely by the intima; left uncovered were two distinct irregular areas about 6 cm. long and

18 mm. wide in their broadest part (fig. 1). Small, very delicate bridges of intima crossed over these areas in several points. The edges of these areas showed many small irregularities which easily could be recognized as the negative image of those of the opposite edges; so apparently the intima had been torn, leaving uncovered the more external coats of the wall of the aorta. These appeared in the ground of the areas described as a gray uneven tissue; no thrombi were seen. In the edges of these areas the intima adhered well to the outer coats except that in some of those parts where the edge was directed straight against the blood stream there was some dissection but not deeper than 5 mm. with the intima floating in water that was allowed to run over the aorta. Apart from that the inner surface of the aorta was smooth and was yellowish except immediately above the aortic cusps where there were some gray areas. In addition, some single small hyaline plaques may be mentioned, which were found especially in the descending aorta.

Of the other organs, only the right adrenal gland is worth mentioning, as it was somewhat enlarged: Its medulla measured 7 mm. in diameter; the cortex seemed not to be hyperplastic. Other findings are stated in the anatomic diagnosis.

Anatomic Diagnosis.—Incomplete spontaneous rupture with aneurysmal dilatation of the ascending aorta; eccentric hypertrophy of both ventricles of the heart; thrombosis of the iliac and femoral veins and of the prostatic plexus; recent hemorrhagic infarcts of the lungs and pulmonary embolism; chronic passive congestion of all organs; anasarca; hypertrophy of the right adrenal gland; small focal caseous tuberculosis of the left superior pulmonary lobe in regression.

Microscopic Observations.—In specimens taken from the myocardium, the lungs, the adrenal glands, the kidneys, the liver and the spleen the macroscopic diagnosis was confirmed by microscopic observation. I shall mention only pertinent details. In the myocardium of both ventricles some focal, not extensive scarring had occurred, with slight lymphocytic infiltration. The branches of the coronary arteries seen in the slides were normal. In the right adrenal gland the medullar substance was hyperplastic, with the characteristic cells appearing in a great number but without pathologic changes. In the kidneys occasional arterioles had a slightly proliferated intima.

Sections of the ascending aorta showed typical and far progressed changes of medionecrosis cystica idiopathica spread all over the media without predilection for the outer coats. There were small areas of muscle necrosis of cloudy appearance, but far more frequent were the pathologic changes of the elastic tissue, with fragmentation and thickening of the fibers, dissolution in granules (which were irregularly scattered about) and complete disappearance of the elastic substance (fig. 2 *A*). Tiny lipoid droplets were frequently found around the elastic fibers. In some parts, muscle regeneration had occurred, with the muscle fibers directed obliquely or even perpendicularly toward the inner surface of the aorta; between such groups of muscle fibers areas of fibrous scar tissue were seen; some of these scars contained wide blood spaces. In other places of severe damage of elastic tissue, sections stained with hematoxylin-eosin showed pale, slightly bluish areas with only few muscle fibers. These areas led sometimes to cystic spaces formed apparently by softening of such pale areas; some of the cysts were filled with slightly basophilic masses coagulated in tiny or coarse filaments; other cysts were filled with cell detritus, free nuclei or amorphous, slightly basophilic mucoid material (fig. 2 *B*). Some of the cysts were round and small; others were larger and consisted of two parts—one round, the other oblong; the latter introduced itself between the layers of the media; the muscle and elastic fibers were simply separated by the oblong part of the cyst but were stopped abruptly by its round part.

Very small intramural hematomas were seen occasionally. Near the adventitia were small blood channels lined by endothelium, separating two layers of the media as did the cysts described in the foregoing paragraph. They opened to round blood spaces about the size of the round cysts (fig. 2 *C*). All these foci of degeneration were free of cellular infiltration. Some vasa vasorum were seen, apparently normal.

The intima showed no noteworthy changes except some insignificant fatty infiltration and hyalinization; it was not thickened. The adventitia was extraordinarily hyperemic and thickened, and was formed by dense fibrous connective tissue.

Transverse sections of the rupture (fig. 3 *A*) showed that the intima and nearly all layers of the media had been torn and that the edges of the rupture had been separated from each other so that a deep ulcer had formed. Some small strips of intima were still found streaming freely in the current of the aorta. In part, also, strips of the torn media must have been streaming, similar to a fluttering flag torn by a strong wind, in the overhanging borders of the ulcer, but later these strips were joined by connective tissue, which developed between them, fixing them in such a way that they appeared in the sections running very irregularly in several directions and stopped abruptly. The borders which were directed straight against

the blood stream had not been torn in such strips, but by a process of dissection the greatest part of the media had been elevated almost entire and had been separated from the adventitia, to which had remained adherent only the external lamellas of the media; but the dissection had not progressed, as the triangular space between the elevated media and the external layers was found to be filled by connective tissue. The latter covered also the ground of the ulcer, where only some scarce remnants of the old elastic and muscular tissue of the media could be recog-

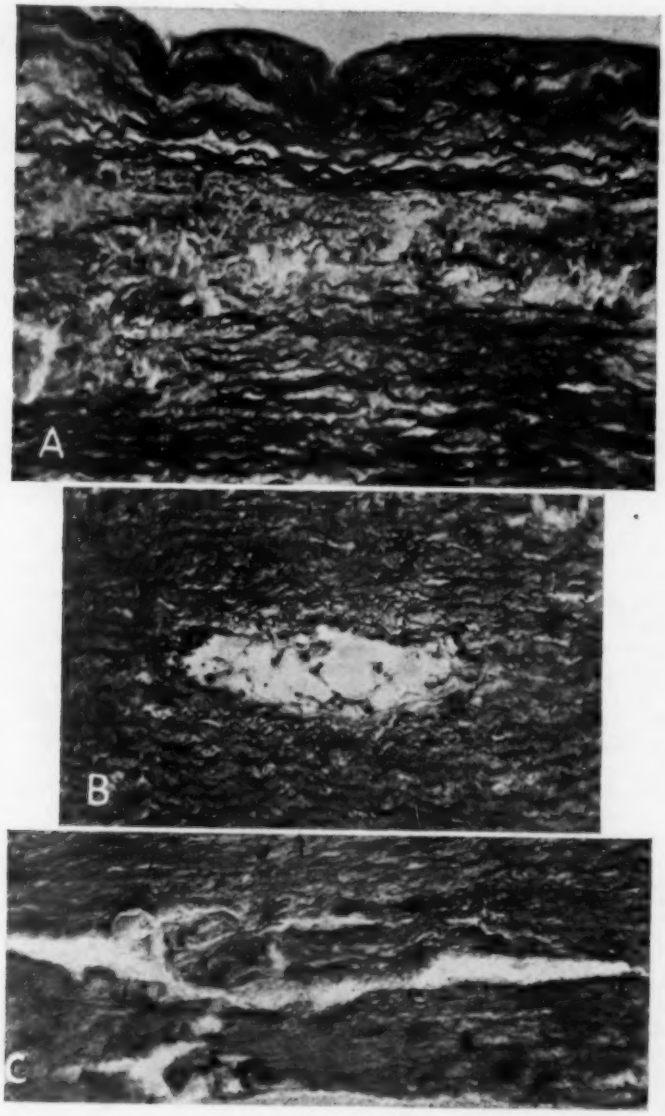

Fig. 2 (case 1).—*A*, photomicrograph showing the damage of the elastic tissue in the subintimal layers of the media; Weigert's stain for elastic tissue; × 130.

B, mucoid cyst containing cell detritus; hematoxylin and eosin; × 110.

C, large blood space near the adventitia lined by endothelium; hematoxylin and eosin; × 83.

nized. This connective tissue formed a thick layer, and one can suppose that if time had permitted it would have filled the whole ulcer. The cells of this connective tissue were round, stellate or oblong, and the direction of the latter seemed to be quite irregular; it appeared to be a very young connective tissue. In some parts its surface was smooth; in others there were cushionlike prominences, and in these some occasional capillary blood vessels were found.

but in general this tissue was avascular. Specific elastic tissue stains disclosed a dense network of tiny elastic fibers which was lacking only immediately under the surface and in the small corners between the torn strips of the old media and was most strongly developed in those parts where in the ground of the ulcer no support was given to the aortic wall by old muscular or elastic tissue. The fibers of this elastic network were most delicate near the aortic surface and showed there no orientation whatsoever. But in deeper layers they appeared somewhat thicker and showed beginning orientation in wavy layers parallel to the surface and running in the same direction as would the elastic fibers in normal media; but in distinction from the latter these fibers were much thinner, not so orderly placed and not separated by muscle tissue. Apparently they were new, regenerated elastic fibers. They could be easily distinguished from the old torn fibers because they were thinner and ran in a functional direction, while the old ones were thick, showed evidence of degeneration, ran in several directions, as if they were placed there accidentally, and stopped suddenly in the place where they were torn. In the combined Van Gieson-Weigert stain the small area under the surface without elastic fibers stained yellow, while the deeper areas showed a red staining ground substance between the elastic fibers.

The descending aorta showed only slight arteriosclerotic changes of the intima; the media and the adventitia were normal.

Case 2.—A woman of 70 years never had been observed by a doctor. During the last two years edema of both legs had developed. Dyspnea did not allow her to work. She had precordial pains radiating to the left shoulder. She fainted frequently. Although all these troubles, especially the dyspnea, were increasing, she still did not ask for medical care. Finally she was admitted to the hospital Nov. 18, 1942, apparently dying. With strong heart-supporting therapy she improved promptly. Physical examination revealed an enlarged heart and a distinct diastolic murmur; other signs of aortic insufficiency were absent. The blood pressure five days after admission was 170 systolic and 110 diastolic. With Kahn's test the serum gave a strongly positive reaction. November 24, at 10:30 p. m. tonic contractions of the muscles of the arms and legs suddenly developed, with severe dyspnea and loss of consciousness; about fifteen minutes later she calmed down and seemed to lie in a profound quiet sleep. Here it must be recorded that the staff on duty that night was extremely busy about another patient in the same ward, a student, who died tragically the following morning; so, unfortunately, the old patient, whose case is reported, was neglected. At 3:30 a. m. she suddenly showed signs of acute dyspnea, was gasping for air, and again had convulsions with expulsion of some foam from the mouth; five minutes later she died.

Necropsy.—The examination was performed twenty-eight hours later. The body was that of an old woman, rather emaciated, with some edema in the lower part of the back and in both legs.

The lungs showed insignificant adhesions to the thoracic wall; they were emphysematous, and the cut surface appeared somewhat hyperemic but dry.

The pericardium was strongly distended by its contents; it was nearly spherical, about 17 cm. in diameter. When the pericardium was opened, the heart appeared embedded in large masses of postmortem coagulums. It was rather small, about the size of the fist of the cadaver. The apex belonged to the left ventricle. The myocardium was firm and reddish and in thickness measured about 20 mm. on the left side and 3 mm. on the right side. The papillary and trabecular muscles of the left ventricle were strongly hypertrophic. All the valves were normal. The intima of the coronary arteries showed hyaline plaques in several places. Near the apex there was a small scarred area in the myocardium of the septum.

When the aorta was opened, a tear was seen in the posterior wall, rather irregular, about 5 cm. long, which ascended obliquely from the supravalvular portion (fig. 3 *B*). Behind this tear and in the vicinity the adventitia was lifted up about 5 mm. by a space filled with blood, which opened on the left side, behind the pulmonary artery, into the pericardial cavity by a small aperture. Beside the intimal tear, at a distance of about 5 mm. there was a groove, slightly curved, 7 mm. long, of reddish ground. As for the rest, the aortic intima showed only hyaline and lipoidic plaques. Otherwise the thoracic organs as well as the cervical ones were normal.

The abdominal aorta, the renal arteries, the inferior vena cava and the adrenal glands were normal. The right kidney was the size of a fist; the enlargement was due to the enormous dilatation of the pelvis. In the back of this balloon-like pelvis an aberrant branch of the renal artery ran to reach what was left of the renal parenchyma; this branch compressed the ureter; by simply cutting this vessel, the pressure was relieved and the content, a clear serous liquid, easily escaped from the pelvis through the ureter. The renal parenchyma was reduced to an area of tissue about 5 mm. thick. The left kidney was somewhat enlarged, measuring 11 by 6 by 4 cm.; its medullary substance appeared congested. The rest of the abdominal organs showed no important changes except chronic passive congestion.

The meninges were extremely hyperemic. In the right hemisphere of the cerebrum, in the area of the basal ganglions and of the capsula interna a large fresh hemorrhage was found which measured about 3 to 10 cm.; the fluid in the ventricles was somewhat hemorrhagic, but no coagulums were found there. The arteries of the base of the cerebrum had sclerotic plaques. The origin of the hemorrhage could not be traced.

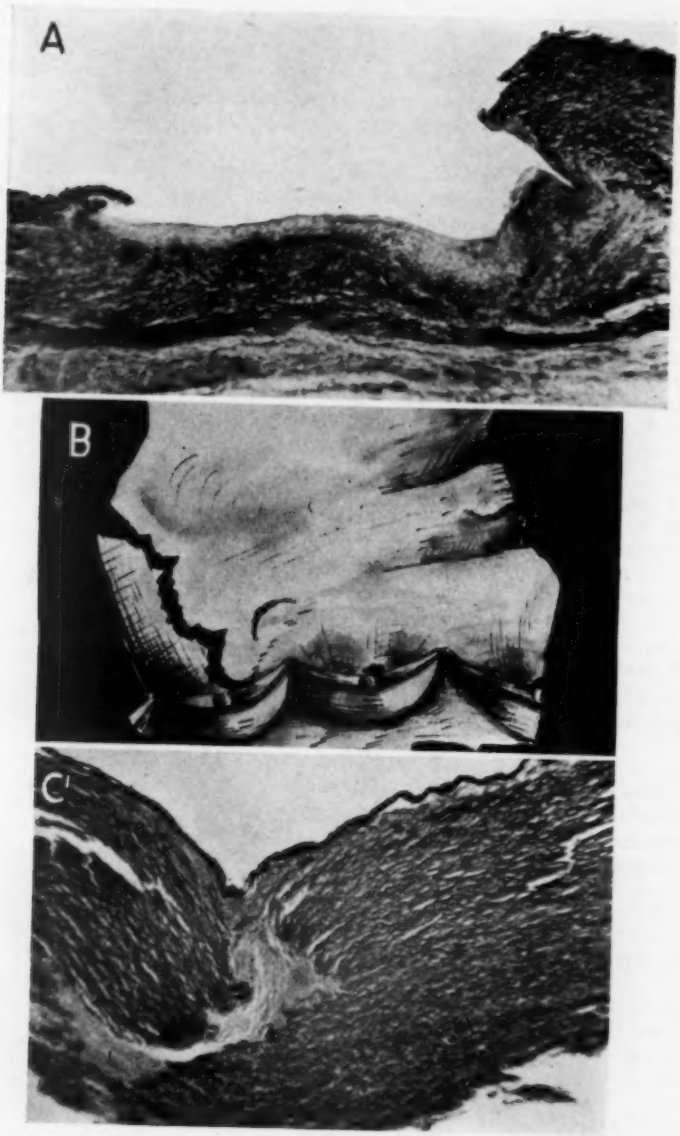

Fig. 3.—*A*, transverse section of the incomplete rupture in case 1, showing on the right the torn stump of the media and on the left part of the intima streaming in the aortic lumen. The ground of the defect has been filled nearly completely by connective and elastic tissue; only very near to the adventitia there are left some remnants of the old media; Weigert's stain for elastic tissue; × 23.

B, drawing of the ascending aorta in case 2 showing the rupture. Note the small curved groove beside the site of rupture; slightly reduced.

C, transverse section of the healed incomplete rupture in case 2; Weigert's stain for elastic tissue; × 43.

Anatomic Diagnosis.—Spontaneous rupture of the aorta into the pericardium; hemopericardium; recent cerebral hemorrhage; generalized arteriosclerosis; hydronephrosis on the left from compression of the ureter by an aberrant branch of the renal artery; hypertrophy of the left ventricle of the heart.

Microscopic Examination.—Most of the aforementioned changes were verified by histologic observation. I need mention only that the difference between the ventricles was most conspicuous in the myocardium, the left showing great hypertrophy of the muscle fibers. There was slight perivascular infiltration by lymphocytes. Some branches of the coronary artery had thickened intima. The scar near the intima looked as one formed after myocardial infarction.

The aorta showed the same changes in the media as were seen in case 1, although developed to a much lesser degree: pale-staining areas, fatty changes, formation of cysts. Several blood channels similar to those of case 1 were seen, which separated the layers of the media. The hematoma which surrounded the site of the rupture was not situated between the media and the adventitia, as supposed in the macroscopic examination, but together with the adventitia a very thin layer of the media had been dissected away from the rest of the aortic wall. In general, no scarring or lymphocytic infiltration was found in the media. The intima showed slight arteriosclerotic changes, especially fatty infiltration. The adventitia was normal; it was neither hyperemic nor thickened.

The groove beside the site of rupture (fig. 3*C*) was covered entirely by intima, which showed there the same change as everywhere: slight fatty infiltration. The lamellas of the media stopped abruptly; it seemed that they had been torn obliquely and that the ends of the torn fibers had separated afterward; but as this rupture had not occurred in the outer layers directly under the place where the inner layers had been torn, the loss of functional media was in no place more than about one-half. The space between the separated ends of the torn media was filled by granulation tissue which contained many blood capillaries, ascending from the adventitia, as well as some fibroblasts, lymphocytes and histiocytes, especially fat macrophages; some collagen fibers had been formed already. As for the rest, between the blood capillaries there was a pale-staining, slightly basophilic ground substance apparently coagulated in delicate filaments.

COMMENT

There can hardly be any doubt that in both cases an incomplete rupture had occurred, in the first case more recently than in the second. In the former the rupture had concerned the intima and practically all the media, so that the adventitia had to withstand alone all the blood pressure; this was possible because it had been thickened previously. That thickening of the adventitia occurs in response to severe medial degeneration was stated first by Rokitansky [2] and has been observed by many authors since (e. g., Rottino [5]).

Apparently the aortic blood in case 1 had been forced to some extent between the layers of the media, yet this did not progress to real dissection; the splintered and separated ends of the lamellas were joined by connective tissue to form a compact tissue. This connective tissue had not yet reached the level of the intima, but if time had permitted, it certainly would have done so. This connective tissue had been formed without the help of granulation tissue; it was practically avascular.

In the second case the medial degeneration was far less advanced, and so no adventitial thickening had occurred; the total rupture in this case was prevented because the external layers of the media had remained intact. Here again the defect was filled by connective tissue, but by means of granulation tissue. The level of the inner surface of the aorta had been reached and the scar was covered by normal intima. Only a shallow groove, perhaps due to beginning retraction of the scar, indicated macroscopically the place of the rupture.

Elastic regeneration occurred in the recent scar at the site of rupture in the first case. It was most pronounced where none of the old elastic fibers remained to withstand blood pressure. The new-formed elastic fibers were very delicate but resembled in their wavy course the old ones. They seemed to develop in close connection with collagenous fibers. That this regeneration of elastic tissue depended

5. Rottino, A.: Arch. Path. **27**:320, 1939.

on the absence of old elastic fibers may be concluded from the fact that in the small spaces between the splintered lamellas of the media which were filled by newly formed connective tissue no such regeneration occurred; neither was it found in the second case, as there the rupture was oblique and in the whole area the old elastic fibers were sufficiently strong.

While the incomplete rupture is healing, the damage of the media in the vicinity progresses. In the second case another rupture occurred, this one complete and followed by sudden death. In the first case, which showed far more advanced changes of medionecrosis idiopathica cystica, it was possibly the thickened adventitia which prevented the aorta from rupturing; the inelastic fibrous tissue of the adventitia yielded gradually to the blood pressure and so the aneurysm was formed. That such an aneurysm from this cause may even look macroscopically exactly like a syphilitic one was demonstrated by Rottino [5]; by histologic observation, of course, the distinction is easy.

My attention was attracted by certain large blood channels, lined by endothelium; they were different from vasa vasorum, as they had no proper muscular wall, being limited simply by the lamellas of the media. Although their exact origin could not be determined, the following theory may well be correct: frequently round mucoid cysts show long projections which separate the medial layers—something like dissecting. Into such a cyst a hemorrhage from a vas vasi may occur; such hemorrhages were seen in one of the cases reported in this article. When no coagulation occurs but, on the contrary, the blood finds a way out, all the necrotic contents of the cyst are washed out by the blood, and the wall will be outlined by endothelium. Of course, such a space will have the same shape as had the cyst, and this was seen in the case described.

The theory that dissection is not the result of incomplete rupture but of hemorrhages from vasa vasorum (as was the general view among the earlier pathologists) is confirmed by these 2 cases, as in both of them incomplete rupture showed no tendency toward progressing dissection even in the presence of severe medial damage, as seen in case 1. But in view of these wide blood channels it seems probable that when the incomplete rupture happens to open one of them the sudden rise of pressure in such an unfavorable mechanical situation might be followed by tearing of the septums between the blood channels, with the appearance of a dissecting aneurysm.

Hypertension is frequently found associated in cases of medionecrosis cystica idiopathica. But its importance in the etiology of this disease, formerly overestimated, is much doubted in the more recent work on this subject. That hypertrophy of the left ventricle as found at necropsy cannot be considered as due to hypertension was made clear by Anderson and Gouley,[6] who found hypertrophy of the heart in all of their 40 cases of dissecting aneurysm. So nothing can be said with reference to blood pressure in the first case; even the slight hyperplasia of the medulla of one adrenal gland cannot be considered proof; in the second case hypertension most probably was present, as demonstrated by the definite hypertrophy of the left ventricle which was not related to the much lesser lesions found in the aorta and by the unilateral hydronephrosis which, according to Nesbit and Ratliff,[7] may cause hypertension frequently. The blood pressure of 170 systolic and of 110 diastolic two days before death might be lower than the pressure which had been present before heart failure occurred.

6. Anderson, E. Q., and Gouley, B. A.: Arch. Path. **28**:421, 1939.
7. Nesbit, N. R., and Ratliff, R. K.: J. A. M. A. **116**:194, 1941.

On the other hand, the importance of a sudden rise of blood pressure, not in causing medial changes but in producing final rupture, is stressed by most authors. Clear examples are the case of Kaufmann [3] (rupture during labor) and of Meyenburg [8] (rupture a short while after sexual intercourse). With reference to this, my second case is most noteworthy because of the coexistence of fresh cerebral hemorrhage and rupture of the aorta. It seems that such an occurrence has not yet been reported in the literature. As the pericardial effusion leads to death within a few minutes by compression of the heart and the big veins, it must be supposed that the cerebral hemorrhage occurred first. The anamnesis, although unreliable and incomplete, indicates that an apoplectic episode happened most probably five hours before death. During this time the intracranial pressure might have risen, stimulating the vasomotor center, followed by a sudden rise of blood pressure above the level of hypertension already previously attained; this pressure caused the diseased aorta to rupture.

SUMMARY

In 2 cases of medionecrosis cystica idiopathica rupture of the inner coats of the aorta occurred but was not followed by dissecting aneurysm: Connective tissue filled the place of rupture. Elastic tissue regenerated where all the elastic support of the media had been destroyed by the rupture.

In one of these cases a complete rupture occurred later in the vicinity of the first one; this accident took place most probably five hours after an extensive cerebral hemorrhage.

8. von Meyenburg, H.: Schweiz. med. Wchnschr. **69**:976, 1939; abstracted, Arch. Path. **30**:818, 1940.

ADVANTAGES OF EGG CULTURE TECHNIC IN INFECTIOUS DISEASES

I. MENINGITIS: (a) PRIMARY ISOLATION OF ORGANISMS FROM SPINAL FLUID; (b) CULTURE OF SPINAL FLUID DURING TREATMENT WITH SULFONAMIDE COMPOUNDS

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The developing chick embryo has been proved an excellent medium for the culture of bacteria.[1] The special value of this living medium has been demonstrated for the culturing of such delicate organisms as Bacillus pertussis, Bacillus influenzae, the meningococcus,[1c, d] the gonococcus,[2] Bacillus tularense, Brucella, Pasteurella pestis [3] and pleuropneumonia-like organisms.[4]

During the past three years we have made studies employing this culture method, applying it particularly to current clinical problems. The present report concerns the use of this procedure for the culture of spinal fluids in cases of meningitis.

The commonly used cultural methods as employed routinely in hospital practice have failed in a disappointing number of instances to yield information concerning the etiologic agent of meningitis.

In 1941 Boisvert and Fousek [5] described an improved method for the isolation and identification of meningococci and gonococci and reviewed methods employed by various workers previous to that date. In a review by Dingle and Finland,[6] dealing with diagnosis, treatment and prevention of meningitis, methods of culture and some of their shortcomings are discussed. Sulkin [7] also emphasized similar difficulties and pointed out that the percentage of positive spinal fluid cultures in cases of epidemic meningitis as reported by a number of authors varies from 33 to 100, with averages of 75 to 95. In an effort to improve averages, Sulkin adapted the mucin-mouse procedure of Miller [8] to the identification of bacteria in the spinal fluid of patients having meningitis, tentatively diagnosed as meningococcic in type. He found this procedure especially valuable when few organisms were present and expressed the belief that the mucin-mouse test is more reliable

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1. (*a*) Goodpasture, E. W.: South. M. J. **26**:418, 1933. (*b*) Goodpasture, E. W., and Anderson, K.: Am. J. Path. **13**:149, 1937. (*c*) Buddingh, G. J., and Polk, A. D.: Science **86**:20, 1937; (*d*) J. Exper. Med. **70**:485, 1939.
2. Bang, F.: J. Exper. Med. **74**:387, 1941.
3. Buddingh, G. J., and Womack, F. C.: J. Exper. Med. **74**:213, 1941.
4. Swift, H. F.: J. Exper. Med. **74**:557, 1941.
5. Boisvert, P. L., and Fousek, M. D.: Am. J. Dis. Child. **61**:710, 1941.
6. Dingle, J. H., and Finland, M.: War Med. **2**:1, 1942.
7. Sulkin, S. E.: J. Infect. Dis. **64**:310, 1939.
8. Miller, C. P.: Science **78**:340, 1933; Proc. Soc. Exper. Biol. & Med. **32**:1136, 1935.

than ordinary cultural methods in establishing the time at which the spinal fluid is free of organisms.

Sacharow [9] undertook a similar study, using egg yolk emulsion as the activating agent in intraperitoneal tests with meningococci in mice and obtained excellent results. However, aside from ease of operation, the new method was not considered superior to the Miller mucin-mouse procedure.

In a recent investigation of cases of purulent meningitis, one of us (A. F. H.[10]) studied the various types with regard to their etiologic agents and on the basis of laboratory reports classified 24 per cent as instances of "sterile meningitis" (no organism isolated by routine bacteriologic methods). It seemed probable that in such instances of "sterile meningitis" organisms were present but failed to grow, for in 45 per cent of these cases questionable bacteria were seen on direct smear of the spinal fluid. To account for the failure to isolate them, possible factors seemed (1) inadequate cultural methods and (2) paucity or poor viability of the organisms.

With these considerations in mind, spinal fluids from patients suspected of having meningitis at the St. Louis Children's Hospital, the City Isolation Hospital and the St. Louis City Hospital were cultured by the egg method simultaneously with culture by the bacteriologic methods used routinely.

As soon as possible after spinal puncture, smears were made from the centrifugate of the spinal fluid, and at the same time 0.1 cc. of the fluid was inoculated directly on the chorioallantoic membranes of eggs that had been incubated eight to ten days, the usual Goodpasture procedure being followed throughout. Simultaneously cultures were made on tryptose blood agar at the St. Louis Children's Hospital and on horse broth blood agar at the City Isolation Hospital and the St. Louis City Hospital.[11] All mediums used contained 5 mg. paraaminobenzoic acid per hundred cubic centimeters. In attempting the primary isolation, microaerophilic and increased carbon dioxide tension methods [12] were used in all instances.

The inoculated eggs were returned to the incubator (39 C.). Eight to twenty-four hours later smears of material obtained by gently scraping the chorioallantoic membranes of these eggs with a sterile platinum loop were stained with Gram's method and examined for the presence of organisms (figure). When organisms were seen in a smear, subcultures from the egg to blood agar were made. When no organisms were seen in a smear, the egg was allowed to incubate for a longer period and was then reexamined. When an egg showed no organisms on smear, subcultures on blood agar were made as a check, but in all such tests bacteria also failed to grow on the blood agar subcultures. When the organism in question was not the meningococcus as shown by primary identification, the special cultural procedures, microaerophilic and 10 per cent carbon dioxide, were dispensed with. In all follow-up subcultures from eggs, microaerophilic and increased carbon dioxide tension methods were used in culturing the meningococci, and aerobic methods were used in culturing pneumococci and influenza bacilli.

PRIMARY ISOLATION OF ORGANISMS FROM SPINAL FLUID

In 52 cases of acute meningitis, the patients ranging in age from 3 months to 65 years, spinal fluid was taken as soon after admission as possible and before

9. Sacharow, B.: Zentralbl. f. Bakt. **147**:175, 1941.

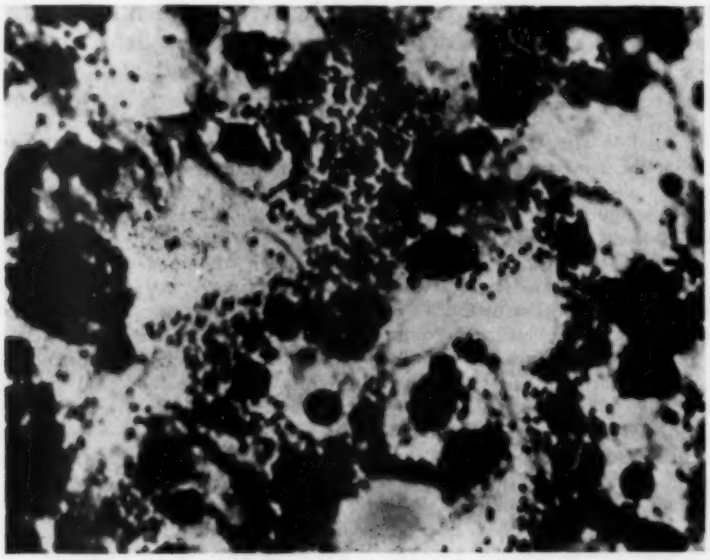
10. Hartmann, A. F.; Wolff, D.; Love, F., and Kendall, B.: J. Pediat. **21**:3, 1942.

11. Formulas: I. At the St. Louis Children's Hospital tryptose broth is made of distilled water, tryptose 2 per cent, sodium chloride 0.5 per cent, disodium acid phosphate 0.3 per cent and paraaminobenzoic acid 5 mg. per hundred cubic centimeters and blood agar is made of 5 per cent defibrinated rabbit blood, tryptose and 2 per cent agar. II. At the St. Louis City Hospital and the City Isolation Hospital horse meat broth agar, containing bactopeptone, human blood 8 to 10 per cent and paraaminobenzoic acid 5 mg. per hundred cubic centimeters, among other ingredients, is prepared according to the method of H. D. Wright. (J. Path. & Bact. **37**:257, 1933).

12. At the St. Louis City Hospital and the City Isolation Hospital the candle method was used; at the St. Louis Children's Hospital, 10 per cent carbon dioxide, Boisvert and Fousek.[5]

initiation of treatment with a sulfonamide compound and was inoculated simultaneously into eggs and on blood agar. For the latter, microaerophilic and increased carbon dioxide culture methods were used. Direct smears of the spinal fluid were prepared in each instance, and a careful search for organisms was made by one of us. In 69 per cent of the cases organisms were seen on direct smear. The agar cultures were observed carefully for signs of bacterial growth, confirmatory smears and subcultures being made. The inoculated eggs were observed at varying intervals, and stained smears of the chorioallantoic membranes were made. Whenever organisms were seen on smear, material from the egg was subcultured on blood agar.

Of the 52 cases included in these comparative studies, 39 were cases of meningococcic infection, of which number 38, when material was cultured on either chorioallantoic membrane or blood agar or both, showed the presence of meningococci. In 1 case a single gram-negative diplococcus was found on smear, but all



Meningococci isolated by primary culture on the chorioallantoic membrane of an egg of spinal fluid taken before the initiation of chemotherapy. The smear was made twenty-four hours after inoculation of the membrane. Gram's staining technic; × 2,000.

cultural efforts to grow the organism failed. In 3 instances growth of meningococci was demonstrated by egg culture when simultaneous agar cultures were negative. Concerning the 35 cases in which meningococci were cultured by both egg and agar methods, in 14, or approximately 36 per cent, meningococci could be demonstrated without question by egg culture twenty-four to eighty-seven hours before definite growth was observed on agar, even when the special cultural methods of reduced oxygen and increased carbon dioxide tension were utilized. Whenever many organisms were seen on direct smear, growth was always good in the egg and usually on agar also. When, however, organisms were not seen on direct smear or were very few, the resulting growth on agar was usually sparse and considerably delayed, while growth in the egg could usually be demonstrated readily within twenty-four hours.

Thus, had reliance been placed entirely on agar cultural methods, our cases of "sterile meningitis" would have amounted to 8 per cent; as it was, with egg

culture methods, the incidence of "sterile meningitis" was only 2 per cent. In the single case in which no organism was cultured, only one extracellular gram-negative diplococcus had been seen on direct smear. The spinal fluid cell count in this case was 24,000. Since the manner of handling this fluid was identical with that employed with the other fluids, the viability of the organism was questioned. It is possible that natural body defenses were unusually efficient in this case. The mild clinical course of the patient seems to support this interpretation.

Spinal fluid was cultured in 4 cases of pneumococcic meningitis. In 3 of the cases gram-positive organisms were seen on direct smear, and in 1 of the 3 direct typing of a type XIV pneumococcus was possible from the first spinal fluid obtained. While in the fourth case no organisms could be found on direct smear, nevertheless within eighteen hours sufficient organisms were present in the inoculated egg to permit typing from the egg of a type III pneumococcus. Only after forty-eight hours' incubation were two small colonies visible on blood agar. Thus, typing of the organism was possible thirty hours sooner when egg culture was employed.

Nine cases of meningitis due to B. influenzae were studied. While in every instance growth of the organism occurred ultimately on agar as well as on egg culture, identifiable growth on the egg required a much shorter time. For example, in 1 case influenza bacilli could be demonstrated within ten hours on egg culture as compared with seventy-two hours on agar culture.

It is of interest to note that on occasions when the organism on primary isolation grew well in the egg and not at all on blood agar, secondary cultures from the egg to agar, carried out in exactly the same manner as before, produced abundant growth in twenty-four hours. This observation indicates that satisfactory growth was possible on the type of mediums employed and that the number and the viability of the organisms implanted on culture are important factors in securing good growth on agar. On the other hand, in the rich medium of the living chick embryo a small number of organisms multiplies rapidly. Presumably weaker organisms and those having more specific growth demands are induced to multiply in the optimum milieu provided by the egg.

CULTURE OF SPINAL FLUID DURING CHEMOTHERAPY

Twelve cases of meningitis were followed during chemotherapy, cultures of the spinal fluid being made daily as soon after puncture as possible. The blood level and the spinal fluid level of the sulfonamide compound used were determined on each daily specimen. Direct smears of the spinal fluid were made and the daily specimen cultured on eggs and blood agar as before. The meningococcus was isolated in 10 of these cases. In every instance except 1, egg culture showed meningococci to be present in the spinal fluid for periods varying from thirty to eighty-seven hours after the blood agar cultures were reported sterile. In the excepted instance there was no growth on agar from the initial spinal fluid sample, compared with heavy growth in the egg within nineteen hours. A second sample, taken twenty-two hours after the initiation of chemotherapy, gave heavy growth in the egg within twenty-two hours and a very scant growth on agar in forty-eight hours. Spinal fluid taken forty-four hours after the initiation of chemotherapy was sterile by both agar and egg methods. It is noteworthy that these results give objective support to the clinical impression that chemotherapy must be continued for a reasonably long period even after definite clinical improvement has been noted. Here again the quantity of inoculum and the number of organisms present must be important factors in obtaining positive cultures by egg when simultaneous

cultures on agar are sterile. After therapy is under way, the chemotherapeutic agent is undoubtedly another factor involved in retardation of growth despite the addition of paraaminobenzoic acid to agar culture mediums. It is possible that in

TABLE 1.—*Results of Culture of Spinal Fluid During Chemotherapy in Cases of Meningococcic Meningitis*

Date	Hours After First Administration of Sulfadiazine*	Direct Smear of Spinal Fluid	Blood Agar Culture Medium			Egg Culture	Subculture from Egg to Blood Agar CO_2 10%	Spinal Fluid		Level of Sulfadiazine	
			Aerobic	Microaerophilic	10% CO_2			Cell Count	% Polymorphonuclears	Blood, Mg. per 100 Cc.	Spinal Fluid, Mg. per 100 Cc.
Patient—A. J., 2 Year Old White Boy											
December 1942											
22	Before	Few gram-negative diplococci	No growth	++ in 48 hr.	+++ in 48 hr.	++++ in 10 hr.	+++ in 24 hr.	4,900	90	0	0
23	13 hr.	No organisms seen	No growth	No growth	No growth	++++ in 24 hr.	++ in 24 hr.	4,000	86	22.2	16.2
24	38 hr.	No organisms seen	No growth	No growth	No growth	+++ in 24 hr.	+++ in 24 hr.	.....	..	48	40.2
26	86 hr.	No organisms seen	No growth	No growth	No growth	No growth	No growth	170	15	26.8	16.6
31	208 hr.	No organisms seen	No growth	No growth	No growth	No growth	No growth	8	0	....	5.7
Patient—B. B., 10 Month Old White Girl											
January 1943											
4	Before	Few gram-negative diplococci	Few minute colonies in 80 hr.	++ in 39 hr.	++ in 39 hr.	++++ in 14 hr.	++++ in 24 hr.	3,800	90	0	0
5	16 hr.	Few gram-negative diplococci	No growth	No growth	No growth	++++ in 18 hr.	+++ in 24 hr.	2,600	90	29.7	20.9
6	40 hr.	No organisms seen	No growth	No growth	No growth	+++ in 19 hr.	+++ in 24 hr.	Bloody tap	..	20.2	16.4
6 Rt. Ventricular Fluid	40 hr.	No organisms seen	No growth	2 very minute colonies in 72 hr.	Few minute colonies in 72 hr.	++++ in 12 hr.	++++ in 24 hr.	Bloody tap	..	....	13.8
7	65 hr.	No organisms seen	No growth	No growth	No growth	++++ in 20 hr.	++++ in 24 hr.	70	70	12.6	7.8
8	87 hr.	No organisms seen	No growth	No growth	No growth	++ in 22 hr.	++ in 24 hr.	50	95	9.6	6.8
9	114 hr.	No organisms seen	No growth	No growth	No growth	No growth	No growth	120	95	9.3	4.7
10	140 hr.	No organisms seen	No growth	No growth	No growth	No growth	No growth	48	100	13.2	7.5
12	152 hr.	No organisms seen	No growth	No growth	No growth	No growth	No growth	.....	..	27.2	17.5
Patient—S. M., 6 Year Old White Girl											
February 1943											
26	Before	Few gram-negative diplococci seen on smear	No growth	No growth	No growth	+++ in 18 hr.	++++ in 24 hr.	10,640	80	0	0
27	20 hr.	No organisms seen	No growth	No growth	No growth	++++ in 24 hr.	++++ in 24 hr.	4,700	80	22.8	14.5
March 1943											
1	68 hr.	No organisms seen	No growth	No growth	No growth	No growth	No growth	120	40	21.7	15.6

* (2-[paraaminobenzenesulfonamido]-pyrimidine).

the egg the chemotherapeutic agent is dissipated and carried away from the site of inoculation by the blood stream of the embryo. At any rate, rapid growth frequently occurs in the egg despite high drug levels in the inoculum (tables 1 and 2).

Similar culture studies were made during chemotherapy in 2 cases of pneumococcic meningitis. One was an instance of type XIV and the other of type III. The presence of organisms in daily spinal fluid samples was demonstrated consistently by egg culture when simultaneous blood agar cultures were frankly sterile. This observation indicates again the importance of continuing chemotherapy for a long enough time.

While it must be emphasized in this work that cultural methods employing blood agar are not to be regarded as optimal, nevertheless these methods of isolation and identification are typical of those used in most hospital bacteriologic laboratories.

TABLE 2.—*Results of Culture of Spinal Fluid During Chemotherapy in Cases of Pneumococcic Meningitis*

Date	Hours After First Administration of Sulfadiazine	Direct Smear of Spinal Fluid	Blood Agar Culture, Aerobic	Egg Culture	Subculture from Egg to Blood Agar	Spinal Fluid		Level of Sulfadiazine	
						Cell Count	% Polymorphonuclears	Blood, Mg. per 100 Cc.	Spinal Fluid, Mg. per 100 Cc.
Patient—B. W., 7 Year Old White Girl with Pneumococcic Meningitis, Type III									
January 1943									
2	Before*	No organisms seen	No growth	++++ in 18 hr.	++++ in 24 hr.	3,900	90	2.2*	Trace*
3	24 hr.	No organisms seen	+ in 48 hr. (2 very small colonies)	++++ in 24 hr.	++++ in 24 hr.	1,070	85	29.9	17.3
4	48 hr.	No organisms seen	No growth	+++ in 21 hr.	+++ in 24 hr.	570	90	31.8	19.7
5	64 hr.	No organisms seen	No growth	No growth	No growth	110	90	45.9	27.6
6	87 hr.	No organisms seen	No growth	No growth	No growth	60	10	35.1	22.0
7	109 hr.	No organisms seen	No growth	No growth	No growth	15	0	38.4	22.8
8	130 hr.	No organisms seen	No growth	No growth	No growth	23	10	30.2	21.6
9	157 hr.	No organisms seen	No growth	No growth	No growth	30	10	23.9	13.4
10	181 hr.	No organisms seen	No growth	No growth	No growth	5	0	18.6	10.7
Patient—C. W. C., 3½ Year Old White Boy with Pneumococcic Meningitis, Type XIV									
February 1943									
8	Before	Numerous gram-positive diplococci	+++ in 24 hr.	++++ in 9 hr.	++++ in 24 hr.	3,160	90	0	0
9	24 hr.	Moderate number gram-positive diplococci	+++ in 24 hr.	++++ in 24 hr.	++++ in 24 hr.	440	85	40.8	17.9
10	48 hr.	No organisms seen	No growth	+++ in 24 hr.	+++ in 24 hr.	500	60	59.6	42.0
11	72 hr.	No organisms seen	No growth	+++ in 24 hr.	+++ in 24 hr.	520	50	37.1	28.2
12	96 hr.	No organisms seen	No growth	No growth	No growth in 96 hr.	260	10	26.8	17.2
13	120 hr.	No organisms seen	No growth	No growth	No growth in 96 hr.	64	0	15.1	8.4

* The child received sulfathiazole (2-[paraaminobenzenesulfonamido]-thiazole) prior to admission to the hospital.

The egg culture method was of value also in proving the presence of viable organisms during a recurrence of meningeal symptoms in a patient having a relapse on the eighth day of illness and after sterile cultures of spinal fluid had been obtained. No organisms were seen on direct smear of the spinal fluid taken on this day, and no growth was obtained by culture on horse broth blood agar. However, within twenty-four hours after inoculation of eggs good growth of meningococci resulted. The spinal fluid cell count was 450, and the sulfamerizine level in the spinal fluid was 2.8 mg. per hundred cubic centimeters.

The accompanying charts (tables 1 and 2) of 3 cases of meningococcic and 2 cases of pneumococcic meningitis are representative of the results obtained with

samples of spinal fluid taken during the course of chemotherapy. It is evident that the presence of viable organisms in the spinal fluid can be demonstrated by the egg method for a considerable time after agar culture methods give negative results and when drug levels in the blood and spinal fluid are high.

SUMMARY

The egg culture method, developed by Goodpasture, is useful for the prompt isolation and identification of the etiologic agent in meningitis. This is true especially in those cases in which no organisms, or doubtful organisms, are seen on direct smears of spinal fluid.

In cases of pneumococcic meningitis prompt isolation is important for the purpose of typing, so that serum therapy, as well as chemotherapy, may be administered early in the course of the illness.

The fact that viable organisms persist, as demonstrated by the chorioallantoic membrane method, in spinal fluids which would be reported sterile by the usual methods of culture and often in spite of high levels of sulfonamide compounds in the blood and spinal fluid, gives support to the clinical practice of continuing chemotherapy for a considerable time after apparent clinical cure.

CHEMICAL BASIS OF INJURY IN INFLAMMATION

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In a recent preliminary communication a brief report was made of observations on the identification of a factor liberated in exudate which is capable per se of explaining the basic pattern of injury as manifested in inflammation.[1]

The inflammatory reaction is characterized by a fundamentally stereopatterned reaction.[2] In the initial phase there is a local disturbance in fluid exchange. This is manifested by an increase in fluid passage, in turn referable to an augmentation in the permeability of the capillary wall. The substance primarily concerned with the mechanism of this phenomenon is leukotaxine.[3] The passage of fibrinogen into the acutely injured area with the subsequent formation of a coagulum in tissues distended with edema, in addition to the ensuing lymphatic blockade, tends to limit the dissemination of the irritant.[4] This capacity of localizing or fixing the irritating material by walling-off the area of inflammation is an important aspect of the inflammatory reaction in regard to immunity, for numerous observations have definitely demonstrated that inflammation plays an important role as the regulator of bacterial invasiveness.[5] The migration of polymorphonuclear leukocytes to the site of an acute injury is likewise referable to the liberation of leukotaxine.[6] The cellular sequence in the development of an inflammation whereby the polymorphonuclear leukocytes usually precede the infiltration of macrophages seems to be conditioned by the hydrogen ion concentration of the exudate.[6] The local acidosis is, in turn, referable to a glycolytic phenomenon, so that true lactic acid acidosis develops in situ.[7] Recent evidences indicate that the injured cells per se are capable of forming dextrose from the products of degradation of the protein molecule.[8] The leukocytosis frequently accompanying inflammatory processes is referable to the liberation of a pseudoglobulin at the site of inflammation.[9] This active protein is absorbed into the circulation, where it is found in measurable quantities.[10] It induces a discharge of immature granulocytes from the bone marrow into the blood stream, thus offering a reasonable explanation for the mechan-

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6. Menkin, V.: Medico-Surgical Tributes to Harold Brunn, Berkeley, Calif., University of California Press, 1942, p. 275; Am. J. Path. **10**:193, 1934; J. Exper. Med. **67**:145, 1938.
7. Menkin, V., and Warner, C. R.: Am. J. Path. **13**:25, 1937. These studies on the role of the hydrogen ion concentration in regulating the cytologic picture in inflammation have recently been confirmed by H. R. Rugiero and C. A. Tanturi (Semana méd. **13**:8, 1942).
8. Menkin, V.: Am. J. Physiol. **138**:396, 1943.
9. Menkin, V.: (*a*) Am. J. Path. **16**:13, 1940; (*b*) Arch. Path. **30**:363, 1940.
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ism of leukocytosis with inflammation.[9a] Available evidence indicates also that this globulin, termed the leukocytosis-promoting factor (abbreviated as the LPF), induces marked hyperplasia in the bone marrow of the granulocytic elements and of megakaryocytes.[11]

The interplay of the aforenamed sequences localizes and disposes of the irritant, thus allowing unhampered regeneration or repair to occur. The fundamental outline in the development of inflammation as sketched is maintained in most types of inflammatory or infectious lesions, irrespective of the irritant. The latter, as well as the anatomic location of the involved area, can modify the final picture; nevertheless, close scrutiny reveals the existence of a basic pattern as described. The presence of some superimposed variables, such, for instance, as the infiltration of lymphocytes or plasma cells, seems to induce an apparent deviation from the fundamental picture. Yet close examination still reveals in many such lesions an initial increase in passage of fluid, the formation of fibrin in distinctly variable amounts and an initial infiltration of polymorphonuclear leukocytes, which are rapidly replaced by macrophages. Neither leukotaxine nor the leukocytosis-promoting factor induces any severe degree of cellular injury. Besides the functions which they perform, these two substances cause relatively little cell damage. Yet inflammation is in itself a manifestation of severe cellular injury. For this reason a search has been undertaken in an endeavor to unravel the basic mechanism of injury in inflammation. This has led, as pointed out in a preliminary communication,[1] to the isolation of an injury factor located in the euglobulin fraction of exudate and termed necrosin. The present communication represents a compilation of the data which have led to the view of the basic mechanism of injury in inflammation. Leukotaxine, the leukocytosis-promoting factor and necrosin are considered as common denominators released by the cell which initially is injured by the irritant. The damaged cell exhibits thus an alteration of its internal chemistry allowing the liberation of various by-products of metabolism. It is these substances (e. g., leukotaxine, necrosin and the leukocytosis-promoting factor) which subsequently initiate the basic pattern manifested in the development of inflammation.

METHOD OF OBTAINING EXUDATE AND PREPARING THE EUGLOBULIN FRACTION THEREFROM

The exudative material was obtained from the pleural cavities of dogs after the injection of about 1.5 cc. of turpentine in each instance.[6] This method of inducing an inflammatory reaction yields appreciable amounts of exudate, which can be readily withdrawn by thoracentesis. In several instances, exudate derived from man was likewise studied.

The euglobulin fraction from the exudate is readily obtained by dialysis against running tap water, which leaves an insoluble residual precipitate in the cellophane tube, or it can be obtained by precipitation with ammonium sulfate at either one-third or one-fourth saturation. After centrifugation, the resulting precipitate is taken up in distilled water and dialyzed against running tap water until free of SO_4. The euglobulin fraction can be readily dried by freezing in a Flosdorf-Mudd apparatus.[12]

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11. Menkin, V.: Federation Proc. **2**:96, 1943; Am. J. Path., to be published.

12. The nitrogen content of the euglobulin fraction (necrosin) averaged on three samples 13.1 per cent. The phosphorus content averaged 0.96 per cent (five determinations). The samples of necrosin utilized were derived from both canine and human exudates. Further information on its chemical and physical properties will be reported in future communications.

POTENCY OF EUGLOBULIN FRACTION (OR NECROSIN) FROM EXUDATE IN COMPARISON WITH OTHER PROTEIN FRACTIONS OF EXUDATE

The severity of an acute inflammatory reaction can be gaged by the rapidity and the intensity with which it induces lymphatic blockade.[13] For this reason the potency of the euglobulin fraction of inflammatory exudate was tested by injecting it into the skin of a forelimb of a rabbit.

As a rule, about 1 to 1.5 cc. of an aqueous suspension of euglobulin was injected intracutaneously in order to test the effectiveness of this fraction in inducing lymphatic blockade. In the skin of the opposite foreleg a solution was injected containing either pseudoglobulin or pseudoglobulin mixed with the albumin fraction, in turn derived from exudative material. The latter procedure served as a control in the testing of the effectiveness of other protein fractions from exudate. After variable intervals, 2.5 cc. of a 1 per cent solution of a diazo dye, trypan blue, in saline solution was injected into the treated areas of skin.[13] Subsequently the extent of lymphatic blockade was determined by the inability of the dye to diffuse to the tributary lymphatic nodes and vessels.[13] On termination of the experiment the sites of cutaneous inoculation and the tributary lymphatic nodes were fixed in 10 per cent solution of formaldehyde for subsequent histologic preparations.

In few instances the euglobulin was brought into solution or at least into a very fine suspension by utilizing physiologic solution of sodium chloride. In this way the effect per se of large aggregates of the insoluble euglobulin material was ruled out. Finally, to control the latter state of affairs even more adequately, an aqueous suspension of euglobulin obtained from normal blood serum was employed in several of the experiments. The euglobulin fraction of exudate can also be dried by freezing, as stated in a foregoing paragraph. In this form as little as 3 mg. of the material, suspended in either distilled water or taken up in physiologic solution of sodium chloride, manifests potent activity. As a rule, however, in the desiccated state 10 to 30 mg. of the euglobulin fraction was injected into the skin of a leg of an animal in order to determine its ultimate effect on lymphatic drainage.

The results of the experiments to establish the relative potency of the euglobulin fraction of exudate are conveniently assembled in table 1. It is clear that the mixed pseudoglobulin–albumin fraction of exudative material failed to induce any lymphatic blockade. Trypan blue diffused readily to the tributary lymphatics as indicated by its presence in the lymphatic nodes or by the recovery of the colored lymph following cannulation of the efferent axillary lymphatic vessel.[13] On the other hand, the dye failed to diffuse readily into the lymphatics draining an area of skin into which the euglobulin fraction of exudate had been injected. The induced lymphatic blockade was striking in the majority of instances. This could be demonstrated as early as about a half hour after the injection of the active protein fraction (rabbit 23-86, table 1). In such experiments the involved region appeared in the gross to be edematous and conspicuously erythematous, with areas of central necrosis varying in extent. This is well exemplified in figure 1. Microscopic examination revealed a dense infiltration of polymorphonuclear leukocytes within a fibrinous reticulum in which occluded lymphatics and small thrombi in vessels were readily found (figs. 2 and 3). Evidently the euglobulin fraction of exudates, recovered either from dogs or from man, is highly injurious, inducing a severe inflammatory reaction containing all the primary elements concerned in the mechanism of lymphatic blockade.[5b]

The deleterious effect of the euglobulin fraction on normal tissue can be detected at an extremely early stage. For instance, ten minutes after this fraction has been introduced into the skin of the abdomen of a rabbit, the collagen bundles may reveal a curiously semihomogeneous ground glass pattern, doubtless

13. Menkin, V.: J. Exper. Med. **50**:171, 1929; **57**:977, 1933.

due to swelling. This is exemplified in figure 4 *A*. At such an early stage there are essentially no leukocytes present. This injurious initial effect on collagenous material is wholly absent after the injection of either leukotaxine or the leukocytosis-promoting factor. Finally, similar effects, though perhaps not quite as

TABLE 1.—*Lymphatic Blockade Induced by Euglobulin Fraction (Necrosin) Derived from Various Types of Inflammatory Exudates*

Rabbit	Interval Between Injection of Euglobulin Fraction and That of Dye		Duration of Inflammation		Presence of Dye in Lymphatics in Either Normal Foreleg or Leg in Which Mixture of Pseudoglobulin and Albumin Had Previously Been Injected		Presence of Dye in Lymphatics in Foreleg with Inflammation Induced by Euglobulin Fraction (Necrosin) Derived from Exudate	
	Hr.	Min.	Hr.	Min.	Lymph of Efferent Lymphatic	Lymph Node	Lymph of Efferent Lymphatic	Lymph Node
21-03	0	18	2	38	++	+	+	+
23-86	0	34	2	55	+	++	Trace	Trace
22-24	0	55	2	32	+	++	Trace	Trace
21-10	3	20	4	53	Trace	Trace to +	0	Trace to +
22-23	13	0	15	43	++	+	Trace	Trace
1*	15	19	17	39	++	++	Faint trace	Trace
22-13†	15	27	17	40	+ to ++	+	0	0
1-82‡	16	23	19	15	++	++	Trace	Trace
21-94‡	17	45	21	43	Very faint trace	+	0	0
1-87§	19	21	22	35	++	+ to ++	Very faint trace	Very faint trace
1-65	19	28	23	31	++	++	0	0
22-47	19	45	24	3	+ to ++	++	0	Faint tract
2-00	20	3	23	12	+	++	Trace	Faint trace
18*	20	10	24	0	++	+++	0	+
3-54	20	19	23	29	+ to ++	+	0	0
22-30*	20	47	23	48	+	++	Faint trace	Faint trace
22-40§*	21	28	25	50	Trace to +	Trace	..	0
17	21	7	23	30	Trace	Trace	0	0
22-17*	21	43	24	53	Trace	++	0	0
22-22‖	22	13	24	28	+	+ to ++	0	0
22-16¶	22	49	26	33	Trace to +	Trace to +	0	0
22-12	22	57	25	9	+++	++	Faint trace	0
22-03	23	45	25	50	+	+ to ++	Trace	0
13§	24	17	28	25	+	++	0	0
22-20*	24	38	27	9	++	++	++	Trace
22-35*	25	18	27	18	++	++	0	0
22-25	41	23	44	0	++	++	0	0
21-41	48	38	51	15	++	++	0	0

* Necrosin was first dried by freezing (Flosdorf-Mudd apparatus); it was then taken up in saline solution and injected into the skin.

† The euglobulin fraction (necrosin) was obtained by precipitation of exudative material at one-fourth saturation with ammonium sulfate; other fractions were "salted out" at one-third saturation with the ammonium salt.

‡ The euglobulin (necrosin) was obtained by dialyzing exudate for only about two to four hours.

§ The euglobulin (necrosin) was obtained from human exudate; the other fractions were derived from canine sources.

‖ The euglobulin (necrosin) was obtained by dialysis of the exudate overnight.

¶ The necrosin was first treated with ether; this is the effect of the ether-insoluble fraction.

intense, are readily obtained by injecting the euglobulin fraction of canine exudate intracutaneously into dogs, an observation indicating that the effect is evidently not referable to the injection of a foreign protein into another species. Furthermore, the pseudoglobulin and albumin fractions of exudate from dogs are wholly ineffective in inducing any appreciable inflammation in rabbits (fig. 5; table 1). Finally, the water-insoluble euglobulin fraction obtained from normal blood serum of dogs fails to induce lymphatic blockade in rabbits. The data of such experiments are recorded in table 2. In brief, these facts definitely seem to show that

Fig. 1.—Effect of necrosin injected into the skin of a foreleg of rabbit *22-25*. The acute inflammatory reaction with areas of central necrosis was drawn about twenty-two hours after the cutaneous injection of 1 cc. of an aqueous suspension of necrosin derived from the exudate of a dog.

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it is only the euglobulin fraction derived from exudate that contains a factor capable of eliciting a fulminating acute inflammation.[14] The observations warrant the inference that in exudative material there is liberated by cells initially injured by an irritant a substance which is either the euglobulin itself or is at least present

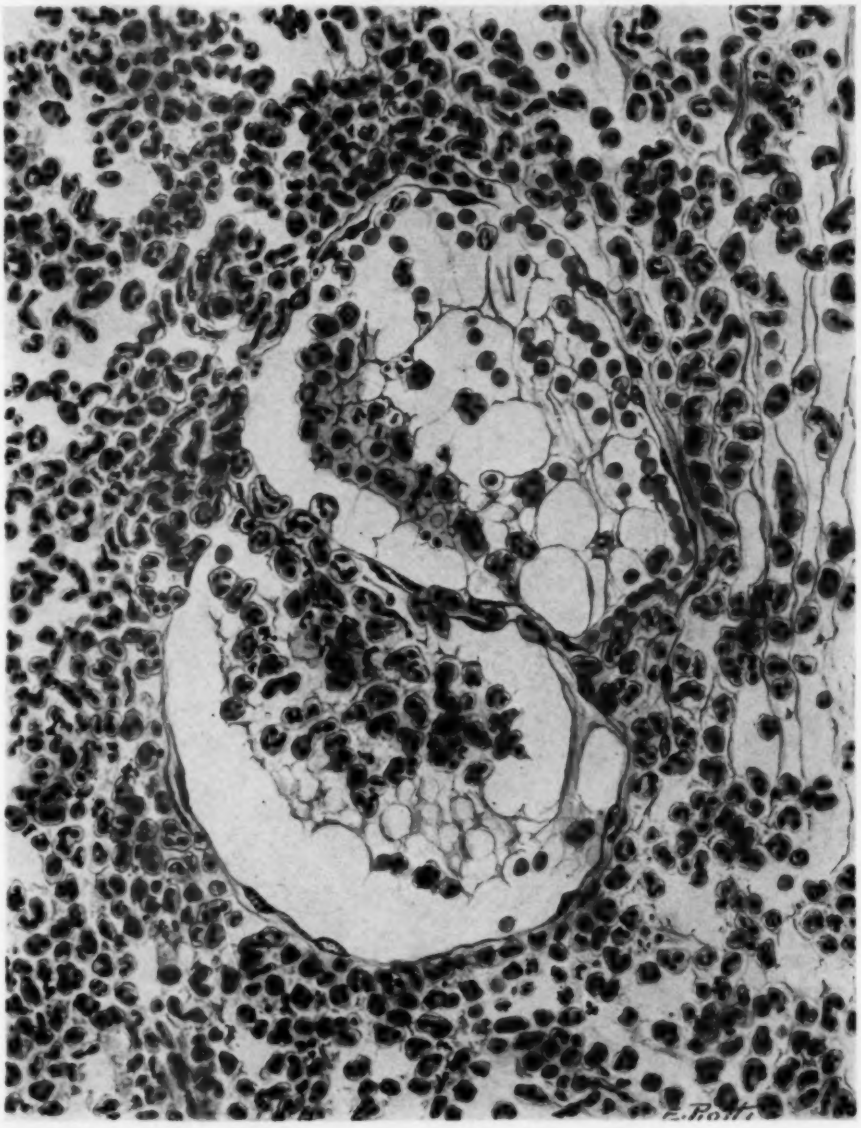

Fig. 2.—Drawing of a thrombosed lymphatic in a zone of dense leukocytic infiltration of a subcutaneous area into which necrosin had been injected about twenty-three hours and thirty minutes previously (rabbit 17, table 1). × about 600.

in the euglobulin fraction of exudate. To this substance, for the sake of convenience, the term "necrosin" has been applied.[1]

14. Although the euglobulin fraction derived from human or canine exudate is highly injurious, the particular fraction from rabbit exudate appears to be relatively weaker or even inactive. This phase is being studied further.

PRESENCE OF NECROSIN IN THE BLOOD STREAM OF ANIMALS WITH A CONCOMITANT ACUTE INFLAMMATION

As mentioned in the foregoing paragraph, the blood serum of normal dogs does not contain a euglobulin fraction capable of eliciting an acute inflammatory reaction when injected into the skin of rabbits. It became of interest to determine whether an active fraction is present in the blood stream of animals having simultaneously an acute inflammatory reaction in the pleural cavity.

The pleurisy was induced with turpentine as previously described. Usually on the following day a sample of blood was withdrawn by cardiac puncture. The serum was treated with

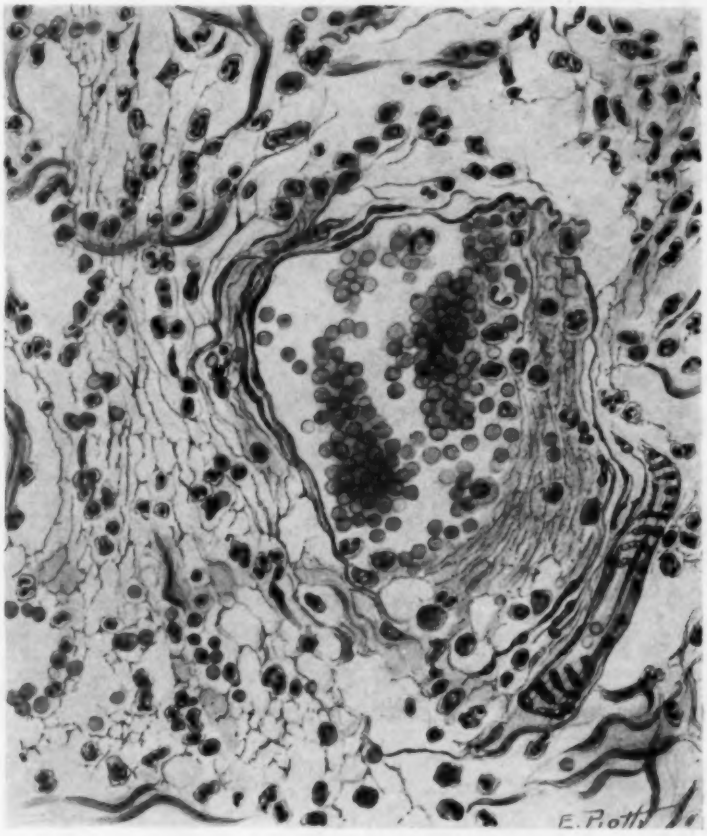

Fig. 3.—Drawing to show the effect of necrosin in inducing a thrombus in a small blood vessel. This is taken from the same section as figure 2 (rabbit 17, table 1). × about 600.

ammonium sulfate at one-third saturation. The resulting euglobulin precipitate was taken up in distilled water and dialyzed against tap water until free of $So_4^{=}$. The supernatant material, containing presumably the pseudoglobulin and the albumin fraction, was likewise dialyzed until free of sulfate ions. These respective fractions were injected into the skin of forelegs of rabbits as described in the previous section.

The results of the experiments appear in table 3. Contrary to the findings with the euglobulin derived from normal serum (table 2), the euglobulin fraction obtained from the blood serum of animals having a concomitant acute inflammation produced in the skin of rabbits an acute reaction leading to lymphatic blockade (table 3). The remaining protein fractions of such serum failed to induce any appreciable degree of inflammation, so that trypan blue injected into the

experimental areas diffused freely to the tributary lymphatic vessels and nodes. The observations seem to indicate that necrosin, or the euglobulin fraction, probably penetrates from the site of an acute inflammation into the circulating blood stream. The significance of this inference is obvious in interpreting the effect of an inflammatory focus on organs located at a distance. The absorption of so-called toxic materials from such areas may well prove to be referable to the passage of necrosin into the circulation from a site of acute inflammation. In this connection it is to be recalled that the globulin termed the leukocytosis-promoting factor has been found to penetrate from an area of inflammation into the blood stream (Menkin and Kadish [10]).[15]

Fig. 4.—*A*, effect of a suspension of necrosin ten minutes after its intracutaneous injection in a rabbit. Note that the collagenous bundles are markedly swollen. This seems to be the first evidence of injury following the injection of necrosin. There is, as yet, no leukocytic infiltration. × 543. In *B* it appears that the euglobulin fraction of normal blood serum is wholly without effect on the appearance of the collagen bundles of the skin even after forty-five minutes. × 543.

15. The following observation substantiates further the view that necrosin probably penetrates from the site of inflammation into the circulating blood. An inflammation in the pleural cavity of a dog (7-93) of about five hours' duration yielded some necrosin in the exudate, whereas this substance was still absent in the circulating blood, where it evidently appeared much later (table 3). This observation definitely seems to indicate that necrosin is formed at the site of inflammation, whence it is subsequently absorbed into the blood stream. Furthermore, the relative absence of leukocytes in such an exudate of a few hours' duration indicates that necrosin is liberated by injured cells in situ and is not necessarily brought to the site of inflammation by leukocytes.

EFFECT OF WHOLE EXUDATE ON LYMPHATIC DRAINAGE

The recovery of necrosin from inflammatory exudate suggests that the untreated exudative fluid is probably injurious per se; otherwise the effect of the euglobulin from exudate on normal tissues may well prove to be referable to the chemical procedure adopted during extraction. For this reason, the whole exudate of dogs was injected intracutaneously into single forelimbs of rabbits. In a previous study it had been shown that exudative material from rabbits induces an acute inflammation in their ears, whereas the serum of the animals is found wholly innocuous.[16] The

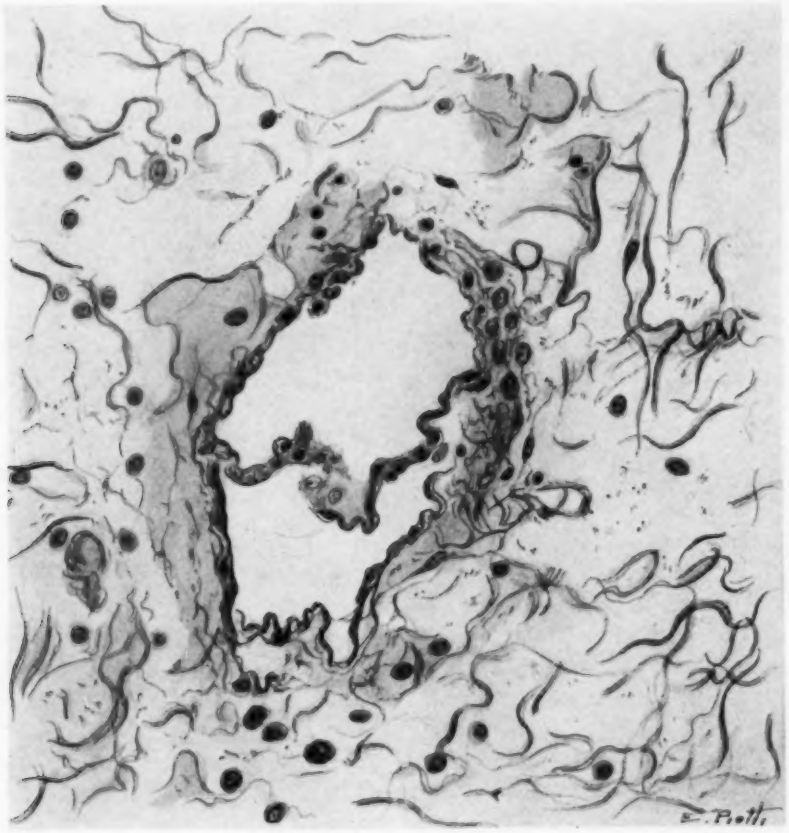

Fig. 5.—Drawing to show the failure of the pseudoglobulin and albumin fractions to induce lymphatic occlusion (rabbit 22-12, table 1). Note also that there is essentially no inflammatory reaction. Approximately × 550.

local injection of canine exudate in rabbits is likewise followed by an acute inflammation, which may perhaps not be as fulminating as the reaction caused by the euglobulin fraction; nevertheless, it is characterized by conspicuous local edema with a variable degree of redness of the skin. Furthermore, the lymphatics are blocked, as indicated by the inability of trypan blue to diffuse to the regional nodes and vessels. The data are compiled in table 4. It is therefore quite clear that the exudate per se is markedly injurious and that, as already shown, the local reaction seems evidently referable to the contained euglobulin fraction or necrosin.

16. Menkin, V.: Cancer Research **1**:548, 1941.

TABLE 2.—*Effect on Lymphatic Drainage of Euglobulin Fraction Derived from Blood Serum*

Rabbit	Interval Between Injection of Euglobulin Fraction and That of Dye		Duration of Inflammation		Presence of Dye in Lymphatics Either on Normal Side or on Side in Which Pseudoglobulin and Albumin Mixture Had Been Injected		Presence of Dye in Tributary Lymphatics of Foreleg with Inflammation Induced by Injection of Euglobulin Fraction Derived from Blood Serum	
	Hr.	Min.	Hr.	Min.	Lymph of Efferent Lymphatic	Lymph Node	Lymph of Efferent Lymphatic	Lymph Node
3-60*	15	30	18	38	++	++	++	++
12-67	18	0	21	0	++	+	++	++
20-70	18	9	20	20	++	+	+	+
3-56	19	45	22	53	++	++	++	++
22-41	21	0	25	57	Trace	+	+	Trace
1-70†	21	32	24	13	++	++	+ to ++	+ to ++
1-93	22	47	26	40	++	++	++	++
1-78*	22	49	26	21	+	+	+	+
5‡	24	32	28	6	Trace	+	Trace	+

* The euglobulin was obtained by dialyzing serum for about four to six hours.

† The euglobulin was obtained by dialyzing the serum over night.

‡ The euglobulin was obtained from serum by precipitating at one-fourth saturation with ammonium sulfate.

TABLE 3.—*Effect on Lymphatic Drainage of Euglobulin Fraction Derived from the Blood Serum of an Animal with a Concomitant Acute Inflammation in the Pleural Cavity*

Rabbit	Interval Between Injection of Euglobulin and That of Dye		Duration of Inflammation		Presence of Dye in Lymphatics Either on Normal Side or Side in Which Pseudoglobulin and Albumin Mixture Had Been Injected		Presence of Dye in Tributary Lymphatics of Leg Inflamed by Injection of Euglobulin Fraction Derived from Blood Serum of Animal with Concomitant Inflammation	
	Hr.	Min.	Hr.	Min.	Lymph of Efferent Lymphatic	Lymph Node	Lymph of Efferent Lymphatic	Lymph Node
3-65	16	20	19	25	++	++	0	Trace
1-53	16	30	19	35	+	++	Trace	Trace to +
14	16	44	21	5	Trace to +	+	0	Trace
22-11	16	57	21	32	+	++	0	0
22-42	19	0	24	0	Faint trace	+	0	0
19	23	23	26	14	++	++	Trace	Trace

TABLE 4.—*Effect on Lymphatic Drainage of Local Injection of Whole Exudate*

Rabbit	Interval Between Injection of Exudate and That of Dye		Duration of Inflammation		Presence of Dye in Lymphatics Draining Area into Which Exudate Was Previously Injected	
	Hr.	Min.	Hr.	Min.	Lymph of Efferent Lymphatic	Lymph Node
22-15*	15	45	18	8	+ to ++	+ to ++
22-60	18	30	21	22	0	Faint trace
22-57	18	47	21	46	0	0
22-62	18	14	22	15	0	0
22-70	19	35	22	15	0	0
2*	19	53	22	58	Trace	Trace
22-61	20	11	23	37	0	0

* Human exudate was employed; in other experiments canine exudative material was utilized.

VARIOUS EFFECTS OF NECROSIN WHEN THIS SUBSTANCE IS INTRODUCED DIRECTLY INTO THE CIRCULATION

Four types of studies with respect to the effect of necrosin introduced directly into the circulation have been thus far undertaken. These include its effect on (1) the temperature of the dog, (2) its influence on the leukocyte level, (3) the injury it induces in some visceral organs and (4) the effect on blood pressure in the cat. The observations may be briefly summarized as follows:

Influence on Temperature.—The introduction of the euglobulin fraction of exudate (i. e., necrosin) into the circulating blood of dogs is soon followed by a definite elevation in temperature. Two experiments are presented to illustrate the point. In each case 10 cc. of the euglobulin fraction of a canine exudate suspended in an aqueous medium was injected intravascularly by cardiac puncture. Thereafter, rectal temperature was recorded at intervals. The results are conveniently tabulated:

Dog 7-40

Time	Temperature	Comment
10:31 a. m.	101 F.	Necrosin injected. From about 11:15 a. m.
1:10 p. m.	105 F.	to about 5:00 p. m. animal repeatedly vom-
2:50 p. m.	106.2 F.	ited, showed evidence of diarrhea with bloody
5:10 p. m.	103.4 F.	stools and in general appeared listless

Dog 7-83

Time	Temperature	Comment
11:27 a. m.	.....	Necrosin injected.
11:30 a. m.	100.9 F.	Dog appeared listless and apathetic for a
2:25 p. m.	103.4 F.	short period following injection of necrosin
4:10 p. m.	103.7 F.	
5:42 p. m.	102 F.	

When necrosin in the desiccated form is taken up in saline solution and introduced into the circulation there develops an appreciable degree of fever. This is exemplified in the following experiment (see also fig. 6):

Dog 7-75

Time	Temperature	Absolute Number of Leukocytes per Cubic Millimeter	Comment
9:35 a. m.	102.2 F.	12,350	
10:55 a. m.	.....	.....	50 mg. of dried necrosin in 10 cc. of saline solution injected into the heart
12:03 p. m.	.....	7,750	
12:18 p. m.	102.3 F.	.....	
12:55 p. m.	104.2 F.	.....	
12:58 p. m.	.....	2,600	
2:20 p. m.	104 F.	.....	
2:25 p. m.	.....	5,550	
2:50 p. m.	104.4 F.	.....	
3:10 p. m.	104 F.	.....	
3:20 p. m.	.....	10,650	
4:40 p. m.	103.2 F.	.....	
4:45 p. m.	.....	17,800	
5:30 p. m.	102.7 F.	19,500	

Following the intravascular injection of necrosin, besides fever, leukopenia invariably develops, which later, concomitantly with a fall in temperature, is followed by leukocytosis. These findings will be discussed presently in further detail.

It is quite clear that after necrosin has been injected into the circulating blood of dogs there develops an appreciable fever which lasts at least several hours. This observation is of significance so far as it may throw light on the factor responsible for the rise in temperature accompanying various inflammatory processes. It is conceivable that the absorption into the blood stream of necrosin from the site of an acute inflammation may likewise induce fever. It is to be noted in this connection that other protein fractions of exudative material have failed to affect the temperature appreciably. For instance, the injection of the pseudo-

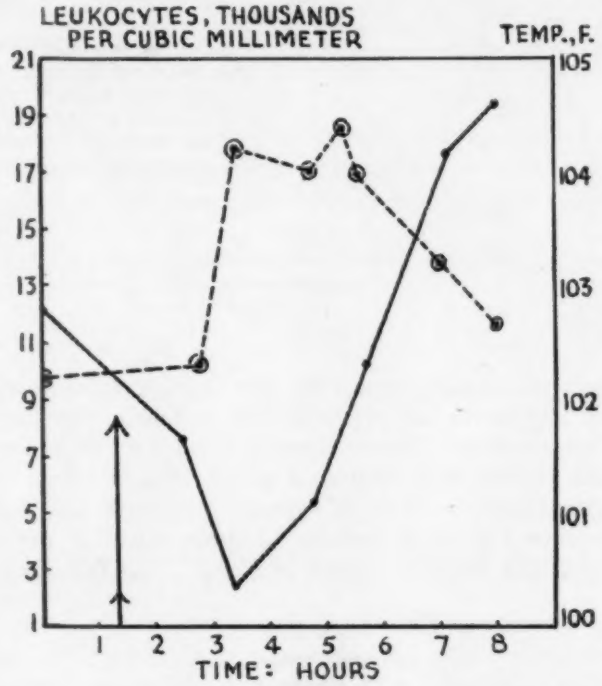

Fig. 6.—Effect of 50 mg. of necrosin injected into the circulation of dog 7-75 on the leukocyte count (solid line) and the temperature (broken line). The arrow indicates the time at which the injection was made.

globulin or the leukocytosis-promoting factor leaves the temperature essentially unaltered. Such an experiment is herewith presented:

Dog 7-80

Time	Temperature	Absolute Number of Leukocytes per Cubic Millimeter	Comment
10:32 a. m.	.....	11,800	
10:55 a. m.	101 F.	.....	
11:00 a. m.	.....	.....	40 cc. of aqueous suspension of leukocytosis-promoting factor injected into the blood stream
12:50 p. m.	101.4 F.	.....	
1:00 p. m.	.....	20,500	
2:08 p. m.	.....	22,950	
2:30 p. m.	101.3 F.	.....	
5:12 p. m.	101.2 F.	.....	
5:21 p. m.	.....	15,150	

The data indicate that the pseudoglobulin fraction of exudate, although capable of raising the level of circulating leukocytes,[9] fails to induce any appreciable fever. Furthermore, the euglobulin fraction derived from normal blood serum is likewise ineffective in causing any elevation of temperature. Such an experiment is herewith presented:

Dog 7-86 (Male)

Time	Temperature	Absolute Number of Leukocytes per Cubic Millimeter	Comment
10:34 a. m.	.....	11,200	
1:00 p. m.	101 F.	.....	
1:20 p. m.	.....	.....	25 mg. of euglobulin from normal blood serum in about 10 cc. of saline solution injected intravascularly. Animal manifested no abnormal symptoms following the injection
2:44 p. m.	.....	9,850	
3:20 p. m.	100.8 F.	.....	
3:43 p. m.	.....	11,750	
4:55 p. m.	101 F.	.....	
5:00 p. m.	.....	11,200	
6:05 p. m.	101 F.	.....	
6:12 p. m.	.....	12,050	

The foregoing facts support further the view that elevation of temperature may well be referable only to the absorption of the euglobulin fraction (or necrosin) from the site of inflammation. This inference is further substantiated by the finding that the euglobulin fraction derived from a sample of ascitic fluid obtained from a patient with cirrhosis of the liver is likewise ineffective not only in inducing lymphatic blockade in a rabbit [17] but also in either elevating the temperature or altering significantly the white cell count of a dog. The following protocol illustrates the point:

Dog 7-78

Time	Temperature	Absolute Number of Leukocytes per Cubic Millimeter	Comment
10:05 a. m.	.....	8,550	
10:33 a. m.	103.2 F.	.....	
10:35 a. m.	.....	.....	65 mg. of euglobulin from ascitic fluid injected intravascularly
11:35 a. m.	102.3 F.	.....	
11:45 a. m.	.....	6,800	
1:05 p. m.	101.5 F.	.....	
1:10 p. m.	.....	11,600	
2:30 p. m.	101.3 F.	.....	
2:35 p. m.	.....	9,900	
3:55 p. m.	101.2 F.	.....	
4:00 p. m.	.....	8,550	
5:47 p. m.	100.7 F.	.....	
5:48 p. m.	.....	11,400	

In brief, the studies indicate that the euglobulin fraction from blood serum or any other body fluid, such as ascitic fluid, seems definitely incapable of inducing

17. Transudates likewise seem to contain euglobulin fractions incapable of inducing lymphatic blockade. Apparently necrosin is recovered only from exudative fluid or from blood serum of animals with a concomitant inflammation (tables 1 and 3).

elevation of temperature in the dog. This is also true of the pseudoglobulin and albumin fractions of exudate. It is quite clear, on the other hand, that the euglobulin fraction (i. e., necrosin) of exudate is highly active in this regard. These studies on the mechanism of the production of fever are being pursued further and will form the subject of future communications.

Influence of Necrosin on the Number of Circulating Leukocytes.—When necrosin is introduced into the circulating blood of a dog, leukopenia develops. The fall in the number of circulating leukocytes is striking and occurs with remarkable promptitude. Within an hour after the injection of about 10 cc. of an aqueous suspension of necrosin or 50 mg. of the dried material taken up in physiologic solution of sodium chloride, a sharp reduction in the number of circulating leukocytes is observed. The leukopenia is apparently not referable to a peripheral redistribution of cellular elements.[18] The evidence for this statement resides in the finding that blood samples obtained by nicking small vessels of the ear compare very well in regard to their white cell content with similar samples obtained by

TABLE 5.—*Effect of Necrosin Injected into the Circulating Blood on the Level of Leukocytes*

Dog	Initial Leukocyte Count	Leukocyte Count About One Hour or Less After Injection of Necrosin	Leukocyte Count 5 to 24 Hours After Injection of Necrosin
7-40	11,850	1,750	48,050
7-43	18,200	1,050	17,900
7-43	13,450	5,550	22,300
7-36	18,650	11,500	29,600
7-43	23,950	1,750	44,550*
7-71	18,450	1,600	18,450
7-43 } Control injection of euglob-	17,900	17,300	23,250
7-36 } ulin from blood serum	14,700	14,550	16,150

* The leukocytosis indicated here was observed only four days later; about six hours after the injection of necrosin, however, there was no appreciable change in the leukocyte count, which was 24,700 white cells per cubic millimeter.

cardiac puncture. The results of a number of experiments are recorded in table 5. When the euglobulin fraction from normal serum is introduced into the blood stream of a dog, there develops essentially no reduction in the number of circulating leukocytes (table 5). This again indicates that the euglobulin of exudative material, or necrosin, has biologic properties that differ from those of the euglobulin fraction derived from normal blood serum.

It is interesting to note that whereas the almost immediate effect on the white cell content of necrosin introduced into the blood stream is leukopenia, this is usually followed several hours later by a sharp rise in the number of circulating leukocytes. The leukocytosis may persist for several days (table 5 and fig. 6). In view of the injurious effect of necrosin on several of the visceral organs, it is conceivable that the leukocytosis may be referable to a secondary effect caused by the liberation of the leukocytosis-promoting factor from various organs, initially injured by necrosin. The possibility of such an interpretation is supported further by the fact that the leukocytosis-promoting factor fails to induce fever when injected into

18. Differential counts were made in a few instances. They indicate relative lymphocytosis. This suggests that the effect of necrosin on the number of circulating leukocytes seems to be primarily on the polymorphonuclear cells.

the circulation and the further fact that the eventual leukocytosis which develops following the earlier injection of necrosin is accompanied by no elevation of temperature. The effect of necrosin in markedly injuring the liver and to some extent the kidneys will presently be discussed.

Injury of Viscera.—The injurious effect of necrosin injected intravascularly in a dog is quite frequently seen in the hepatic and to some extent in the renal

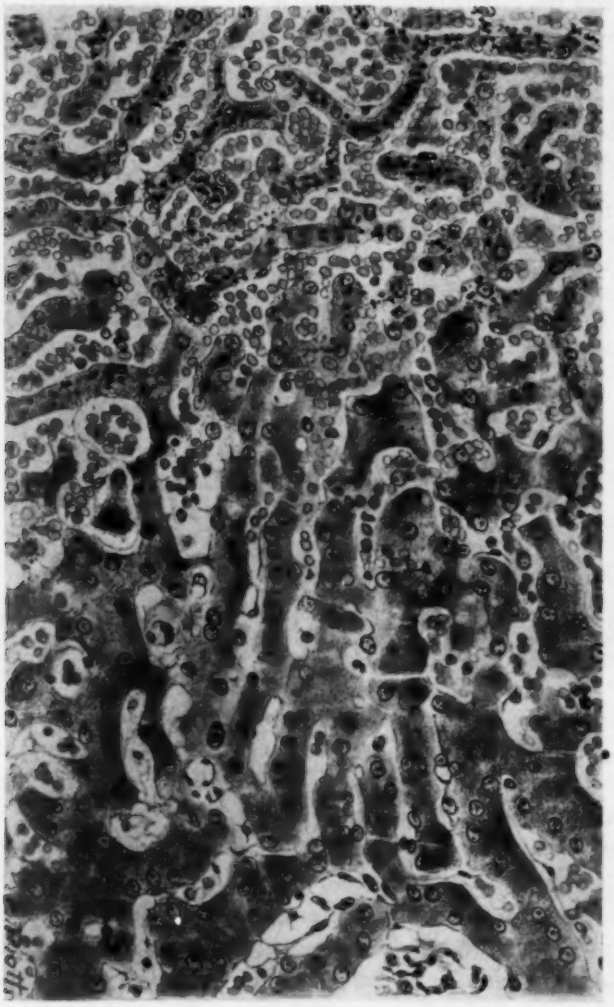

Fig. 7.—The effect of a single intravascular injection of 10 cc. of a saline suspension of canine necrosin on the liver of dog 7-83. The animal was found dead on the following day. Note the destructive effect on some of the hepatic cords. The nuclei seem to be replaced by granular stipplings, which stain intensely with hematoxylin. It is conceivable that these granules represent residual nuclear material. The sinusoids in such areas are markedly congested. On the right the hepatic cords appear to be fairly normal. × 540.

structures It is true that other tissues may be affected but their involvement is not as constant a feature as that of the liver and the kidneys. The liver in the gross may show several irregular areas of whitish discoloration suggestive of necrosis. At

times the areas may appear yellowish or even hemorrhagic. Microscopic examination reveals a picture which perhaps can be described best as that of focal necrosis, although at times the injury seems to be more characteristically zonal. The involved area may show an absence of nuclei, the normal hepatic cords being replaced by a

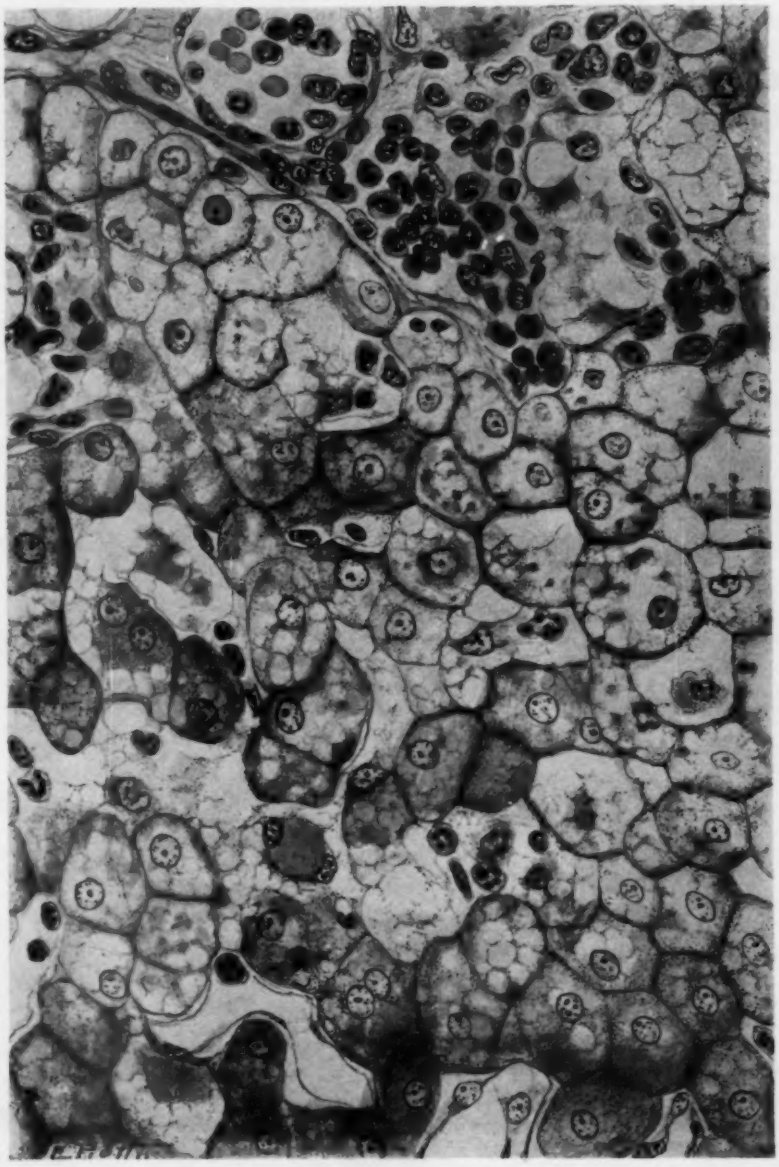

Fig. 8.—The liver of dog 7-45 more than thirty hours after the intravascular injection of 10 cc. of an aqueous suspension of necrosin recovered from a human pleural exudate. Note the conspicuous vacuolation in many of the hepatic cells. There is also a focus of leukocytic infiltration. A week prior to the administration of necrosin this dog had received an intravascular injection of the euglobulin fraction derived from normal blood serum. The latter injection induced no appreciable blood changes, whereas the present injection of necrosin was followed by severe leukopenia which on the next day was followed by leukocytosis. ×760.

strewn mass of intensely black stained granules (fig. 7). These granules fail to take the iron stain. It is suggestive that they may represent the remains of disintegrated nuclear material. Interspersed in such areas of parenchymatous injury are found considerably congested sinusoids. In other cases the impairment may reveal itself in the form of irregularly outlined areas of vacuolation. The hepatic cells appear as if they were loaded with fat granules, whereas other sections of the liver appear

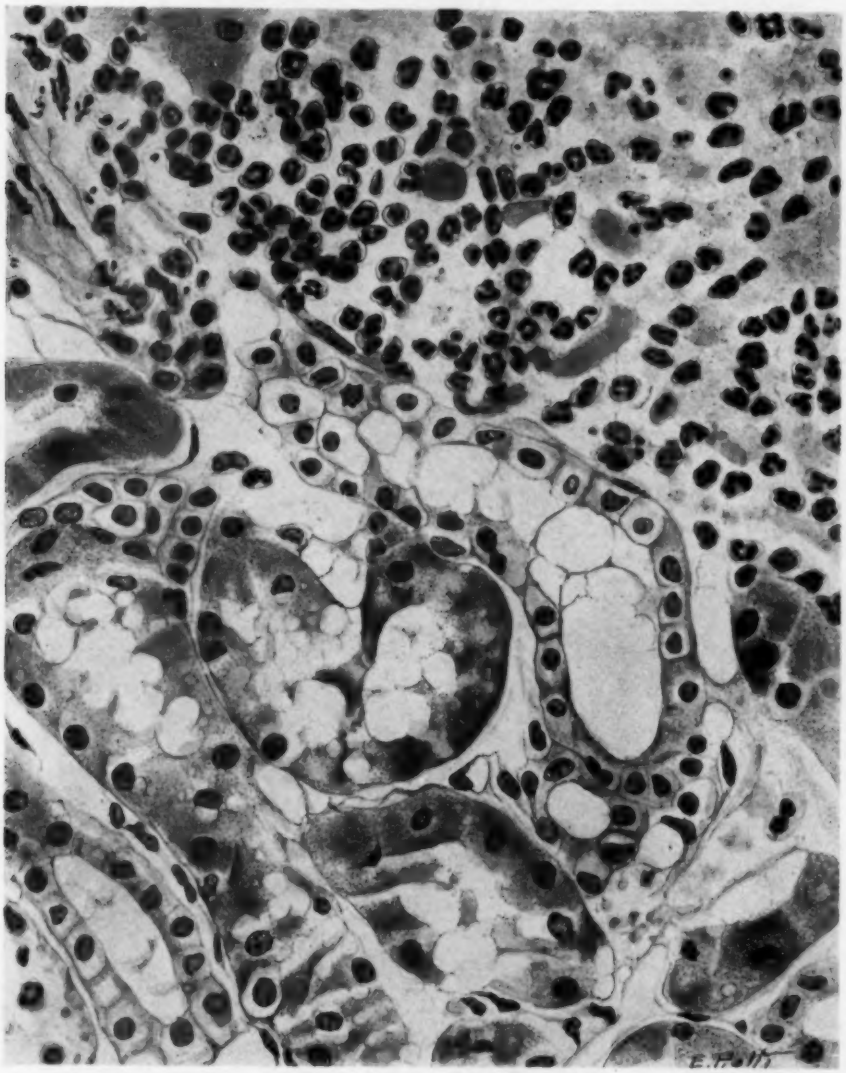

Fig. 9.—Section through a kidney of dog 7-40 about seven hours after a single intravascular injection of 10 cc. of a suspension of necrosin obtained from another dog's exudate. This animal (i. e., dog 7-40) displayed marked leukopenia (table 5) and a sharp elevation in temperature from 101 F. to 106 F. in slightly over four hours. The leukopenia was succeeded by marked leukocytosis (table 5). Note in the drawing the focus of polymorphonuclear leukocytic infiltration with very few mononuclear cells. The tubules likewise show, in many instances, prominent vacuolation of the lining epithelial cells with irregular contour of their surface. × 760.

unaffected. Adjacent to such injured foci one may encounter small clusters of leukocytes (fig. 8).[19]

The renal capsule strips readily, frequently revealing on the surface of the organ a few discrete miliary abscess-like lesions. The tubules may appear somewhat paler than usual. Staining for fat (scarlet red) shows that this seems to be referable, at least in part, to an excess amount of lipoid staining material in a number of the tubular structures. Occasionally, fairly well delineated foci of infiltrating polymorphonuclear leukocytes, interspersed with some macrophages, may be discerned on microscopic examination (fig. 9). The tubular epithelium frequently appears markedly vacuolated, and the contour of the cells lining the lumen may be hazy and irregular (fig. 9). The glomeruli may be moderately packed with cells, some of which appear to be polymorphonuclear leukocytes.

The stomach and the intestine throughout its length may reveal scattered areas of congestion. The mesenteric lymph nodes may at times be enlarged and succulent. In 1 instance exudative-like fluid was found in the pericardium and in both pleural cavities on the day following an intravascular injection of 10 cc. of an aqueous suspension of necrosin. The animal had succumbed during the night. As

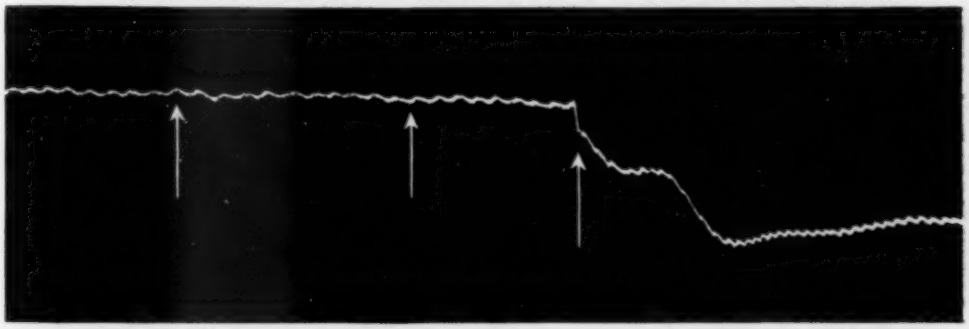

Fig. 10.—Effect on the blood pressure of necrosin when injected into the circulating blood of a cat (no. 4). The introduction into the femoral vein of 49 mg. of necrosin from a human exudate at 5:47 p. m. (first arrow) failed to alter the blood pressure, which remained at 170 mm. of mercury (second arrow). When, at 5:49 p. m. (third arrow), about 4 mg. of histamine dihydrochloride was injected, there followed a sharp drop in blood pressure. Very much smaller doses of canine necrosin (e. g., 13 mg.) likewise failed to alter the blood pressure in the cat.

19. In a preliminary communication dealing with the present study[1] it was pointed out that prolonged dialysis of the otherwise noninjurious leukocytosis-promoting factor or of blood serum may cause the formation in the cellophane tube of a large precipitate, which in turn is capable of inducing an acute inflammatory reaction. The early work of Chick[20] on the conversion of pseudoglobulin to euglobulin following prolonged dialysis (i. e., thirty-six hours or more) has suggested the possibility that such formed euglobulin possibly possesses toxic properties. In this connection it is interesting to note that one such fraction of the leukocytosis-promoting factor was dialyzed for five days in the course of its purification. At that time a conspicuously large precipitate was found in the cellophane tube. This material introduced into the circulation of a dog induced marked leukopenia. The white cell count fell from an original level of 13,350 to 1,950 per cubic millimeter. The white cell count never regained its original level and the dog was found dead on the day after the injection of the material. The liver in the gross presented an interesting picture, with its irregularly yellowish areas suggestive of fatty degeneration. Microscopically, the hepatic cords were found markedly damaged, particularly around the central portions of the lobules. The nuclei were in large part absent, leaving in place of the hepatic cords large empty spaces containing loose strands of apparent fibrous-like material. The picture was somewhat reminiscient of an early cirrhotic lesion.

a rule, however, the dogs survived the administration of the material in the doses employed. For the purpose of study the animals were killed a day or two later. The bone marrow was, as a rule, not particularly striking. It was either normal appearing or slightly aplastic. If the animal was allowed to live for several days, during which the original leukopenia was followed by leukocytosis, the marrow might show, although not necessarily, varying degrees of mild hyperplasia.

Effect on Blood Pressure.—In view of the injurious potency of necrosin it is quite natural to inquire concerning its possible role in surgical or traumatic shock. It is possible that the term "shock" represents the resultant of several profound changes due to severe injury. This may involve a combination of intense local inflammation and all its attending circumstances with a superimposed neurogenic reaction. A fall in blood pressure has been frequently described in both clinical and experimental shock. For this reason it became of interest to determine the effect of necrosin on blood pressure. A cannula was introduced into the carotid vessel of a cat under anesthesia induced with dial. One to several cubic centimeters of an aqueous suspension of necrosin was then injected into the femoral vein. There resulted no drop in blood pressure in several such experiments. The record of one of them is shown in figure 10. It is conceivable that, although necrosin is evidently not concerned with the reduction in vascular tension manifested in shock, nevertheless it still may play a significant role as one of the liberated and injurious substances concerned in the general syndrome of this condition.

COMMENT

The observations reported in this communication definitely indicate the presence of a substance which is, or at least is associated with, the euglobulin fraction of inflammatory exudate and which offers a reasonable explanation for the basic mechanism of injury in the development of inflammation. This substance, termed necrosin, reproduces the gross and the microscopic appearance of an acute inflammatory reaction. Its injurious effects are readily gaged by its capacity to induce rapid lymphatic blockade. The euglobulin of normal blood serum or the other protein fractions of exudative material are inactive in inducing any such reaction of injury. Only prolonged dialysis of the euglobulin fraction of blood serum for thirty-six hours or more, or of the leukocytosis-promoting factor, may favor their conversion into a euglobulin fraction sufficiently toxic to induce an acute inflammatory reaction. This is an interesting fact in view of the observations of Chick,[20] who reported a number of years ago that after prolonged dialysis a certain amount of pseudoglobulin is converted into euglobulin. One wonders whether such converted material may not be analogous to necrosin or the active euglobulin recovered from exudate. At any rate dialysis of normal serum for only several hours or of the recovered precipitate obtained by treating normal serum at one-third saturation of ammonium sulfate yields, in contrast to the findings with exudate, a wholly inactive fraction (table 2). The observations strongly suggest that initial injury of cells by an irritant disturbs or alters their internal chemistry. The result is the formation of various by-products which in turn act as common denominators in the development of the basic pattern of inflammation. Such newly formed substances have already been identified in earlier studies. They consist of leukotaxine, the leukocytosis-promoting factor and now necrosin. Their interplay clarifies considerably the understanding of the fundamental mechanisms concerned in inflammation. The phenomenon of inflam-

20. Chick, H.: Biochem. J. **8**:404, 1914.

mation is considered as a manifestation of severe local injury in which the cellular metabolism is doubtless markedly deranged. Various other substances are likewise liberated by damaged tissue. For instance, it has recently been found that local gluconeogenesis seems to be a consequence of cell injury.[8] In brief, sufficient data brought up in the present and earlier communications support the point of view just outlined that the initial injury induced by an irritant in turn initiates a completely new type of cell chemistry. This state of affairs can adequately explain the various mechanisms concerned in the development of the inflammatory reaction.

In connection with the foregoing studies it is necessary to recall the enormous literature on the syndrome of shock. The earlier observations of Cannon [21] led him to the belief that there probably is a toxic substance absorbed from the area of severe injury which is in large part responsible for the condition. Krogh [22] expressed a similar view, namely, that traumatic shock is referable to the formation of toxic substances which are ultimately distributed throughout the body by the circulating blood. Vaughan and his collaborators [23] stressed the importance of the products of protein hydrolysis as toxic substances. More recently Best and Solandt [24] concluded that a substance or substances liberated by injured tissues may be concerned in traumatic shock. Many years ago Turck [25] demonstrated that products of disintegrating tissues are injurious when injected into animals. This observer also expressed the belief that wound shock is referable to absorption of toxic material from damaged tissue. Finally, Moon and Kennedy [26] induced shock in dogs by introducing finely minced muscles into the peritoneal cavity. The findings presented by me in this paper indicate that necrosin is found in the exudate at the site of an acute inflammation, from which it seems to be absorbed into the circulating blood.[27] The possible relation of necrosin to one of the substances postulated to exist in shock is merely suggestive and therefore requires further study. The fact that necrosin fails to depress blood pressure would presuppose that the fall in vascular tension is referable to another entity.

The finding that necrosin induces leukopenia has been utilized in the further purification of the leukocytosis-promoting factor. This entails a preliminary elimination of the euglobulin fraction or necrosin from exudate.[11] That necrosin per se induces leukopenia is interesting and suggests that it may yet prove to be of great significance in the understanding of various leukopenic manifestations in some well known infectious processes.

Studies to determine the possible chemical relation of leukocidin and of other toxic products of micro-organisms to necrosin loom as attractive features and are therefore being studied. Various other biologic properties of necrosin, such as that of hastening blood coagulation (mentioned in an earlier report [1]) will form the subject of subsequent communications. Studies on the further chemical purification of this substance are also in progress.

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26. Moon, V. H., and Kennedy, P. J.: Arch. Path. **14**:360, 1932. Moon, V. H.: Shock: Its Dynamics, Occurrence, and Management, Philadelphia, Lea & Febiger, 1942.

27. Although necrosin is not present in the serum of normal animals, it can at times be recovered from blood serum showing an extensive degree of hemolysis. This suggests that the material may be liberated by the injured red blood corpuscles.

It is perhaps of interest to comment briefly on the capacity of necrosin to induce fever. A number of years ago Grafe [28] attributed fever to the irritative action of the decomposition of injured tissue and of bacteria. Barbour [29] has reviewed the various theories on the mechanism of fever. Some investigators have referred fever to the stimulation of a "heating center" and the depression of a "cooling center" in the central nervous system.[30] Others have expressed the belief that fever is referable to a deficit of free water in the body.[31] Without going further into these various views, which seem to have little experimental support, it is conceivable that the absorption of necrosin from the site of an acute inflammation by inducing an elevation of temperature, may prove to be an observation of value in studying and clarifying further the important mechanism of fever.

SUMMARY AND CONCLUSIONS

Inflammatory exudates from man or dogs contain a substance which is markedly injurious to the cutaneous tissue of rabbits and to some extent to that of dogs. The introduction of this substance induces an acute inflammation characterized by intense redness, edema, a variable degree of superficial necrosis and lymphatic blockade. The material is either the euglobulin of exudate or is at least associated with that particular protein fraction of exudate. Its presence at the site of inflammation offers a reasonable explanation for the basic pattern of injury in the development of the acute inflammatory reaction. In contrast, neither leukotaxine nor the leukocytosis-promoting factor induces any severe degree of injury when introduced into the skin of rabbits. For the sake of convenience the potent injurious euglobulin fraction of exudate has been termed necrosin. Studies are in progress in an endeavor to purify further this newly identified substance.

Necrosin is absent from normal nonhemolyzed blood serum, but it is detectable in the serum of an animal with acute pleural inflammation. In contrast to blood serum, the whole exudate per se is injurious, since it is able to induce an acute inflammation with lymphatic blockade. The pseudoglobulin and albumin fractions of exudate are incapable of causing any such tissue reaction. The observations therefore strongly suggest that the euglobulin fraction, or necrosin liberated presumably by cells initially injured by an irritant, contains the active principle of exudate which is largely responsible for the basic pattern of injury in the development of inflammation.

Necrosin fails to depress the blood pressure of the cat. Its injection into the circulating blood of dogs is followed by marked leukopenia, elevation of temperature and damage to some of the visceral organs, particularly the liver and to some extent the kidneys. The leukopenia is eventually replaced by leukocytosis.

The observations on the presence of necrosin at the site of an acute inflammation are discussed from the standpoint of an alteration of cellular chemistry. The consequence of such a derangement is the release or the formation of various basic substances from injured cells. These may include leukotaxine, the leukocytosis-promoting factor and necrosin. These by-products of a disturbance in cell metabolism in turn act as common denominators and as such are in large part responsible for the fundamentally stereopatterned picture discerned in the development of the inflammatory reaction. The possible role of necrosin in explaining various leukopenic manifestations, as well as its significance in understanding the mechanism of fever with inflammation is pointed out.

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HYPERTENSIVE DISEASE OF THE BRAIN

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CINCINNATI

The involvement of the brain during the course of arterial hypertension has been sufficiently emphasized by a number of observers. In spite of the frequent occurrence of cerebral symptoms complicating arterial hypertension, the changes in the nervous system seldom have been described in detail.

In 1940 I called attention [1] to the vascular alterations in hypertensive encephalopathy. The histologic changes which were regarded as typical were hyaline degeneration of the walls of the smallest arterioles associated with diffusely scattered small circumscribed areas of recent and old softening and foci of minute hemorrhages in the vicinity of blood vessels. The cerebral vascular lesions were considered to be similar to those found in the kidneys of patients with arterial hypertension. The view was expressed that the hyaline degeneration of smaller blood vessels was a special form of vascular alteration and therefore should be considered separately from the vast group of changes typical of arteriosclerosis.

This presentation is based on a detailed study of the changes in the brain in 25 cases of hypertensive encephalopathy. The purpose now is to describe the characteristic histologic features of hypertensive encephalopathy and to discuss the relationship between them and the vascular changes typical of arteriosclerosis.

TWO ILLUSTRATIVE CASES

CASE 1.—A 38 year old Negro woman had seven admissions to the Cincinnati General Hospital between 1932 and 1942. The presence of arterial hypertension was first noted in 1936, when the blood pressure was 225 systolic and 145 diastolic. Her main complaints were occipital headache, dizziness and ringing in the ears. The heart revealed an accentuated and tympanitic second sound, a muffled and almost inaudible first sound and no murmurs.

In 1937 the patient complained of palpitation, orthopnea and disturbance of sleep. The heart disclosed sinus arrhythmia and a tympanitic second sound at the aortic area. There were marked signs of heart failure. The urine has a specific gravity of 1.020, 20 white blood cells, traces of albumin and sugar. The blood pressure was 230 systolic and 160 diastolic.

In 1941 the patient was readmitted complaining of sharp substernal pain lasting two to three minutes, orthopnea, oppression in the chest, edema of the ankles, frequency of urination and nocturia. The heart disclosed gallop rhythm and moderate enlargement to the left and down but no murmurs. The blood pressure was 200 systolic and 137 diastolic. The ocular fundi contained tortuous vessels; the arteries were of "silver wire" appearance and the veins congested. The specific gravity of the urine was 1.022. It contained no albumin or casts.

After the last admission, Sept. 7, 1942, the patient had progressing dyspnea, pain in the right lower region of the chest and generalized weakness. There was a gallop rhythm of the heart, with a systolic murmur at the apex and over the aortic area. The blood pressure was 204 systolic and 140 diastolic. Death occurred December 25.

The clinical diagnosis was hypertensive heart disease with chronic myocardial failure and acute cystitis.

The gross changes observed in the postmortem examination were marked myocardial hypertrophy with cardiac dilatation and congestive failure (pulmonary congestion and bilateral hydrothorax), acute lobular pneumonia of the right lung and moderate fatty degeneration of the kidneys.

From the Laboratory of Neuropathology, Cincinnati General Hospital, and the University of Cincinnati College of Medicine.

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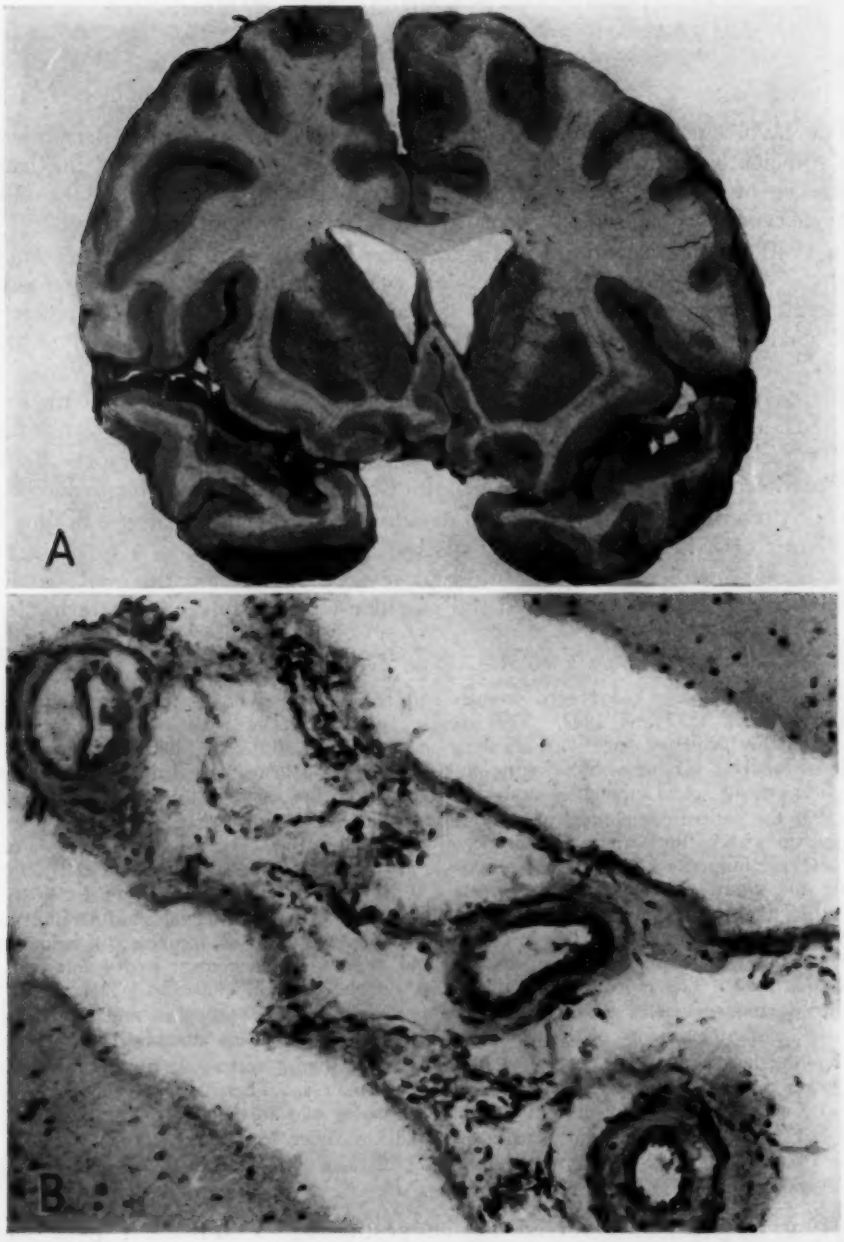

Fig. 1 (case 1).—*A*, atrophy of the sylvian region with slight dilatation of the lateral ventricles. *B*, early stage of hyaline degeneration of the smaller vessels. Note the circumscribed or diffuse subendothelial deposits of hyalin with narrowing of the lumen. Cresyl violet stain; × 165.

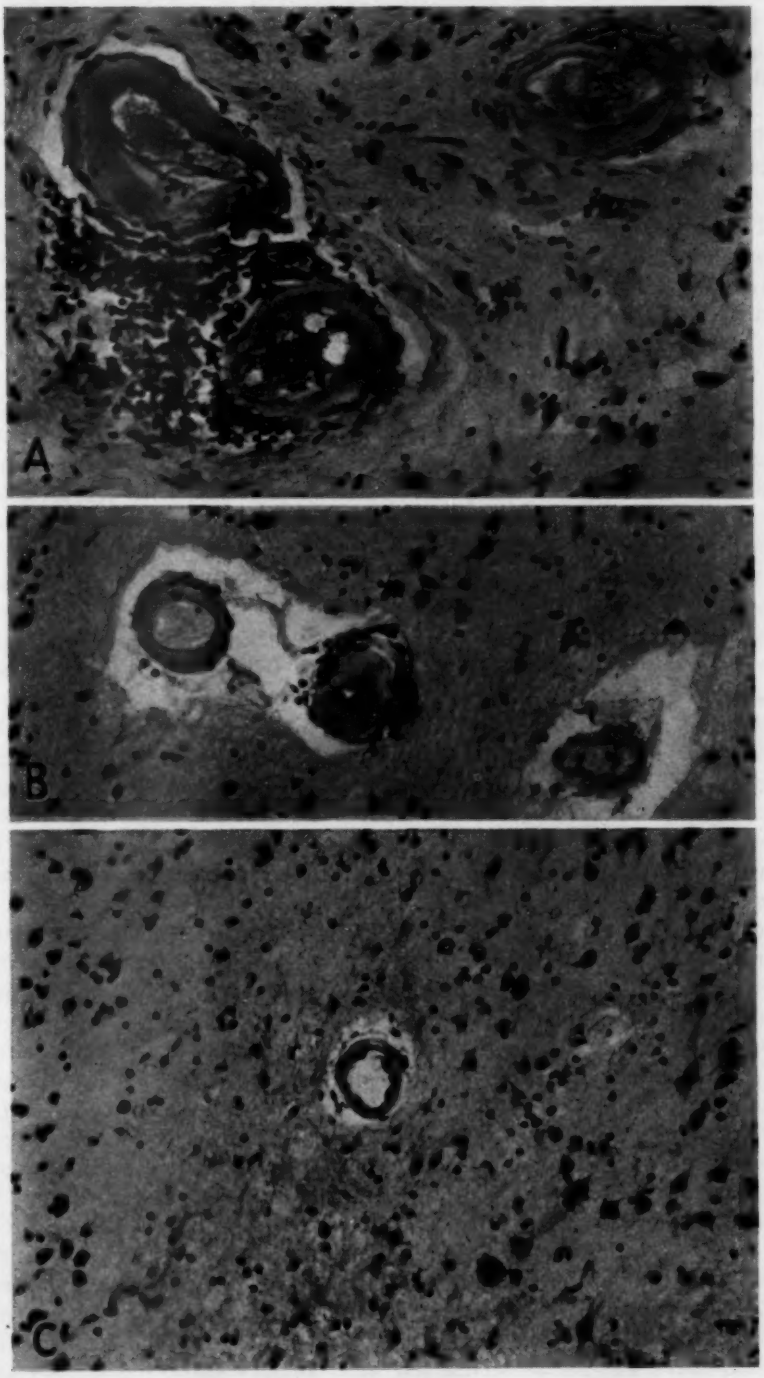

Fig. 2 (case 1).—*A*, far advanced hyaline degeneration of the walls of the vessels. Note the perivascular accumulation of cells. Cresyl violet stain; × 165. *B*, hyaline degeneration and thickening of a small blood vessel. Note the almost complete obliteration of the vessel's lumen. Cresyl violet stain; × 165. *C*, circumscribed area of focal perivascular destruction of nerve cells. Cresyl violet stain; × 165.

Gross examination of the brain showed atrophy of the tissue adjacent to the sylvian fissures and some dilatation of the lateral ventricles (fig. 1 *A*). The blood vessels at the base were soft and of rather large caliber.

Sections were taken for microscopic study from several areas of the cerebral cortex, from the basal ganglions and from the cerebellum. The examination revealed the chief alterations to be in the vascular system and in the parenchyma of the brain.

The main histologic alterations of the vascular system are illustrated by figures 1 *B* and 2 *A*, *B* and *C*. The majority of the arterioles and capillaries exhibited widespread diffuse thickening, homogenization and hyaline degeneration of the entire wall, fibrous thickening and proliferation of the periadventitial fibrous tissue and localized areas of perivascular lymphocytic infiltration.

The hyaline degeneration was the most striking and the most frequent alteration of the walls of vessels. It was usually confined to the smallest arterioles. In the early stage it consisted of a subendothelial accumulation of homogeneous acidophilic substance which usually appeared as an infiltration or expansion of the ground substance of the subendothelial connective tissue. It stained yellow with the hematoxylin and Van Gieson stain and pale blue with cresyl violet. The deposition of hyalin varied according to the stage of the process. Sometimes, especially in the early stage of the degenerative process, it was to be seen in the form of a circumscribed subendothelial accumulation with displacement of the lumen of the vessel to an eccentric position (fig. 1 *B*). In the more advanced stages the hyalin formed an annular collar of irregular thickness involving the entire wall of the vessel (fig. 2 *A* and *B*). The majority of the arterioles and capillaries with hyaline degeneration disclosed extremely narrowed lumens, and some of them appeared to be almost obliterated (fig. 2 *B*). These alterations were occasionally associated with secondary changes of the media (thinning and homogenization). They were never or extremely seldom complicated by fatty or mucoid degenerative changes. Necrosis or plaque formation was not observed.

There was striking fibrous thickening of the periadventitial connective tissue, a change which was most marked around the capillaries and which is similar to the condition known as "pericapillary fibrosis."

Perivascular cell accumulation (seen in fig. 2 *A* and *B*) varied greatly as to character and severity. In some areas the perivascular cells could be identified as lymphocytes; they were usually mixed with adventitial cells, which in some instances showed distinct proliferation.

Changes of the nerve tissue, secondary it is thought to the vascular alterations, consisted of circumscribed areas of focal destruction of nerve cells, disseminated throughout the cortical ribbon and usually confined to the nerve tissue adjacent to the smaller blood vessels (fig. 2 *C*). These devastated areas usually contained no cellular elements except a few glial nuclei. In addition there were circumscribed areas of tissue necrosis characterized by complete destruction of all tissue elements, which were replaced by a large accumulation of fat granule cells. These lesions may be classified as an acute form of tissue softening obviously resulting from insufficient blood supply. Small areas of glial scar formation were disseminated throughout the gray and the white matter. These focal lesions represented repair and replacement of destroyed nerve tissue by glial fiber and cell proliferation. Occasionally there were to be seen a few macrophages harboring blood pigment.

Case 2.—A 48 year old Negro woman was brought to the Cincinnati General Hospital in a delirious state. She was first seen at the hospital in 1941 when she complained of blurred vision of six months' duration. For one year she had had frequent occipital headaches, tinnitus, disorder of memory, dyspnea on exertion, frequency of urination and nocturia. Pertinent physical findings at this time were a blood pressure of 210 systolic and 130 diastolic, blurring

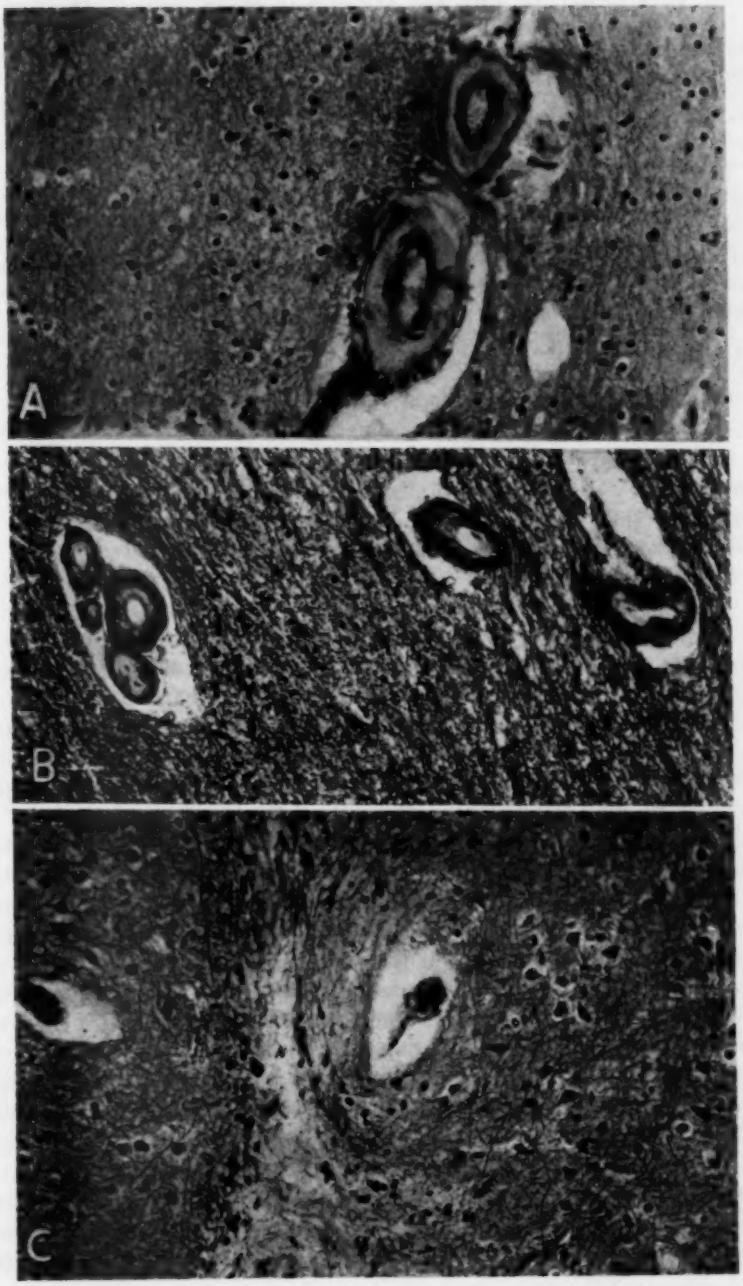

Fig. 3 (case 2).—*A*, hyaline degeneration, homogeneous appearance and thickening of the wall of a blood vessel. Cresyl violet stain; × 165. *B*, fibrous thickening of the walls of blood vessels. Bodian silver impregnation method; × 165. *C*, old circumscribed area of tissue destruction. Bodian silver impregnation method; × 165.

of the nasal margins of the disks, and narrowing and tortuosity of retinal vessels with punctate and flame-shaped hemorrhages. The patient was followed intermittently for about eight months. She continued to have the same symptoms, and hypertension was always present.

Beginning about September 1942 and extending until the time of her final admission, March 8, 1943, she had frequent spells of dizziness and falling without loss of consciousness. Recurrent headaches and blurred vision continued. During the last six months her memory had become increasingly worse; she was frequently disoriented and confused and had experienced sense deceptions. There was unsteadiness of gait, and for three months she had shown urinary and fecal incontinence.

On March 8 she responded incoherently on painful stimulation. Cheyne-Stokes breathing was observed. The pupils reacted sluggishly to light. The margins of the optic disks were blurred on the nasal side, the vessels of the retinas were tortuous, and old and recent hemorrhage and exudate were seen. There was resistance to passive movement of the neck, and there was slight weakness of the left upper extremity. The plantar responses were extensor on the left. The blood pressure was 220 systolic and 130 diastolic; the pulse rate 140.

The course was persistently febrile, the temperature ranging between 100 and 102 F. The patient's mental condition fluctuated between stupor and active delirium. On the eighth hospital day the temperature rose to 103 F., and consolidation at both lower lobes of the lungs was found. The patient lapsed into coma and died on November 17.

Extensive confluent lobular pneumonia and arteriolonephrosclerosis were found at the gross examination.

The brain was generally small, and there was slight irregularity of the convolutional surface because of scattered small areas of atrophy. The leptomeninges were slightly thickened. The vessels at the base were thin walled and contained a few scattered sclerotic plaques. Coronal sections revealed diffusely scattered, circumscribed small foci of cystic softening. One area of cystic destruction of limited size was to be seen in the right caudate nucleus and one in the region of the right internal capsule. A more recent small softening was to be seen in the left cingulate gyrus. Small foci of recent hemorrhage were to be seen in the left thalamus and hypothalamus.

Sections were taken for microscopy from several areas of the cerebral cortex, the basal ganglions, the pons and the cerebellum and were stained with hematoxylin and eosin, cresyl violet and Bodian silver impregnation. The examination revealed vascular changes and disseminated lesions of the parenchyma similar to those described in case 1. A detailed description will therefore be omitted. The vascular alterations (fig. 3 *A* and *B*) were diffuse thickening, homogenization and hyalinization of the walls of arterioles and capillaries. The majority of the smaller blood vessels had extremely narrowed lumens, and there was fibrous thickening of the entire wall (fig. 3 *B*). The hyalin formed an annular collar of irregular thickness. This often resulted in a homogeneous appearance of the entire wall of the vessel (fig. 3 *A*). Some of the blood vessels were surrounded by perivascular cuffs. No necrosis or plaque formation was observed.

The principal alteration in brain tissue consisted of numerous small areas of softening, chiefly confined to the vicinity of the smaller blood vessels which had extremely narrowed or completely obliterated lumens. Older foci of destruction were represented by circumscribed areas of glial scarring (fig. 3 *C*). In more recent lesions there was a large accumulation of gitter cells which harbored debris of tissue and pigment granules. In addition there was to be seen diffuse ischemic nerve cell degeneration throughout the cortical ribbon of both hemispheres.

COMMENT

These cases present typical examples of the lesions of the brain in hypertension. It appears that the most constant and uniform alteration consists in a specific vascular process characterized by hyaline degeneration and fibrous thickening of the arterioles and capillaries. Alterations of the parenchyma were observed in all

cases, consisting of scattered small areas of recent softening with complete destruction of the nerve tissue and replacement by large masses of gitter cells, circumscribed foci of glial scar formation, numerous perivascular hemorrhages, massive hemorrhage and diffuse or localized edema of the brain.

The presence of the small foci of altered nerve tissue contiguous to the tremendously narrowed or completely obliterated arterioles seems in itself to be sufficient indication that the tissue changes were secondary to the described arteriolar lesions.

Considerable difference of opinion exists as to the classification of the morphologic alterations of the smaller blood vessels. Some investigators consider them as the arteriolar counterpart of simple arteriosclerosis.[2] Most investigators, however, seem to focus their attention on the hyaline deposits in the walls of the vessels rather than on structural alterations of the vessels. It is generally known that hyaline degeneration may be observed frequently in blood vessels with changes typical of arteriosclerosis. This apparent similarity has led to the conclusion that the arteriolar alterations observed in cases of hypertension belong to the great group of changes of arteriosclerosis.

In spite of their vague morphologic resemblance, there are indications that there are significant differences between the two conditions. These might be detailed as follows: 1. In arteriolar alterations associated with hypertension a diffuse hyaline degeneration of the *entire* wall is the most striking and predominant feature. These changes are never, or extremely seldom, complicated by the variety of secondary changes such as necrosis of the hyperplastic intima, fatty degeneration and sclerotic plaque formation typical of arteriosclerosis. 2. Although intimal hyalinization does occur in both conditions, in arteriosclerosis the intima usually shows marked hyperplasia associated with necrosis, changes which are infrequently seen in hypertensive arteriolar alterations. 3. Marked degeneration of the internal elastic membrane, represented by swelling, disruption and dispersion of fibers with eventual complete necrosis, lesions pathognomonic for the arteriosclerosis, are not frequently seen in the arteriolar hyalinization associated with hypertension. 4. In arterial hypertension the vascular changes are usually confined to the arterioles and capillaries and may be present without sclerotic changes of the larger blood vessels, as illustrated in case 1. It should be stressed, however, that a combination of sclerosis of the larger vessels with the arteriolar hyalinization typical of hypertension does occur frequently.

From these points of differentiation it seems proper to conclude that the arteriolar changes in hypertension ought to be considered as a special form of vascular alteration and should be separated from the vast group of arteriosclerotic changes under the heading of hypertensive hyaline arteriolopathy.

In support of this conception a few corroborative points will be mentioned. Keyes and Goldblatt [3] described vascular changes in the eyes of dogs and monkeys with persistent hypertension following experimental constriction of the main renal arteries. These changes were somewhat similar to those which have been described as hypertensive hyaline arteriolopathy. The arterioles of the choroid, the ciliary body and the iris exhibited "widespread degenerative changes with the evidence of collagenous and hyaline degeneration of the wall of the vessel." Some of the vessels were surrounded by cuffs of lymphocytic infiltration. Arterioles with similar structural alteration were also present elsewhere in the bodies of these animals.

2. Rühl, A.: Deutsches Arch. f. klin. Med. **156**:129, 1927. Wirtz, H.: Beitr. z. path. Anat. u. z. allg. Path. **97**:219, 1936. Hiller, F.: Arch. f. Psychiat. **103**:1, 1935. Moritz, A. R., and Oldt, M. R.: Am. J. Path. **13**:679, 1937.

3. Keyes, J. E., and Goldblatt, H.: Arch. Ophth. **29**:812, 1938.

In the experimental studies of Nedzel [4] on the vascular response to pitressin in the central nervous system of dogs, structural alterations of the smaller blood vessels similar to those of hypertensive encephalopathy were described. The vascular changes in dogs which were given injections of pitressin at the rate of one a week for a period of from one to three years were described as follows: "Some of the blood vessels had highly thickened walls and some were obliterated. Especially numerous were thickenings of the walls of blood vessels, particularly of the smaller ones." In addition to these vascular changes, Nedzel observed small focal areas of destruction of nerve tissue and perivascular demyelinized patches. The vascular changes were regarded by Nedzel as the end result of angiospastic phenomena induced by the action of pitressin. This brings to mind the cerebral symptoms associated with arterial hypertension, in which periodic and fluctuating clinical symptoms are frequently observed—for example, transient palsies of the extremities and fluctuating speech disturbances. These clinical manifestations are explained most satisfactorily as due to relatively unstable vasomotor regulation of the cerebral circulation. They are obviously the result of functional vascular disturbances, possibly angiospastic phenomena. It appears that one is justified in suspecting that the characteristic arteriolar changes found particularly in association with hypertension may be due to functional vascular disturbances of prolonged duration or of repeated occurrence. There is evidence enough to presume that the cerebral damage of hypertension may result from vascular disturbances which are at first functional and after a period structural.

SUMMARY

The characteristic histologic features of hypertensive encephalopathy observed in 25 cases are reported and 2 cases are described in detail.

Typical vascular alterations, confined to the arterioles and capillaries, were observed in all cases and consisted of hyaline degeneration and fibrotic thickening of the walls associated with narrowing or obliteration of the lumens. These arteriolar changes should be considered as a special form of arteriolopathy typical of hypertension and different from those found in the usual case of arteriosclerosis. A working hypothesis presumes that the characteristic arteriolar change may be due to functional vascular disturbance of prolonged duration or of repeated occurrence.

Changes in the parenchyma of the brain consisted of diffusely scattered, circumscribed small foci of old and recent softening, perivascular hemorrhage, massive hemorrhage and diffuse or localized edema of the brain. The view is expressed that the alteration of the brain tissue is secondary to the arteriolar lesions.

4. Nedzel, A. J.: Vascular Spasm: Experimental Studies, Urbana, Ill., University of Illinois Press, 1943.

EXPERIMENTAL APPENDICAL MUCOCELE, MYXOGLOBULOSIS AND PERITONEAL PSEUDOMYXOMA

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It was demonstrated in a previous paper [1] that mucocele of the appendix could be produced experimentally in the rabbit. Pseudomyxoma peritonei followed in nearly every instance if the mucocele was allowed to remain long enough in the peritoneal cavity. The pseudomyxoma varied from organizing deposits of myxomatous material, either encysted within the layers of a mesentery or plastered over peritoneal surfaces, to encapsulated tumor-like implants attached to various mesenteries, to the parietal peritoneum or to organs within the peritoneal cavity. In several instances the material aspirated from a mucocele or a secondary implant was capable of initiating the formation of "tumor" when deposited in the peritoneal cavity of another, hitherto unused rabbit. The latter finding led to the impression that the material from a mucocele might contain an active substance, possibly viable cells, which could be propagated by transfer from animal to animal. In the previous paper the question was raised whether the transplanted "tumors" arose from epithelial cells originally derived from the appendical mucosa or from serosal cells irritated by the mucous deposits. The belief that viable epithelial cells were responsible for the transplantability of the material was in line with the views expressed repeatedly in the literature by various authors who dealt with the subject of peritoneal pseudomyoma.

The majority of writers have accepted the view that mucosal cells are intermixed with the escaped mucus and that these become implanted on the peritoneal surfaces. All these authors have accepted the appendical origin for these cells on the basis that cuboid and columnar epithelial cells are frequently found in sections of involved peritoneum. They differ, however, in their evaluation of such findings. Fraenkel (cited by Michaelson[2]) expressed the opinion that epithelium originating in the appendical mucosa becomes implanted on peritoneal surfaces but that it does not have proliferative qualities. Jefferies [3] expresses the view that "pseudomyxoma peritonei is caused by transplantation and continuous growth of low and high columnar cells cast off from the lining of a mucocele." Burkhardt (cited by Michaelson [2]) suggested the possibility that the epithelial cells associated with peritoneal adhesions in pseudomyxoma might be endowed with proliferative qualities and might form autonomous growths biologically related to cancer. On the strength of his clinical investigations and of the experimental work of Naeslund,[4] Michaelson contended that the appendical epithelial cells may become implanted in the abdomen and may proliferate. In his opinion it is important to differentiate between epithelium-containing pseudomyxoma and the variety that

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1. Grodinsky, M., and Rubnitz, A. S.: Surg., Gynec. & Obst. **73**:345, 1941.
2. Michaelson, E.: Acta chir. Scandinav. **68**:37, 1931.
3. Jefferies, J. W.: Ann. Surg. **96**:215, 1932.
4. Naeslund, J.: Upsala läkaref. förh. **34**:1, 1928; Arch. Path. **7**:148, 1929.

does not contain epithelium. The latter, according to him, is always a noncancerous clinical entity, while in the former one has to reckon with the changes in the cellular elements, and the prognosis is often doubtful although it is not a question of histologic cancer. Hentz [5] assumed that the appendical cells carried away into the peritoneal cavity continue to function and to produce pseudomucinous fluid. "Otherwise," he remarked, "how can the occasional presence of enormous amounts of gelatinous material be explained?" Yet he expressed the belief that "present-day knowledge is not sufficient to answer the question whether the pseudomucinous fluid in the abdominal cavity is inflammatory or neoplastic." According to this author, the fact that spontaneous regression of pseudomyxoma has been observed is a point in favor of the supposition that the condition is inflammatory in nature.

As to the relation of pseudomyxoma to cancer, comparisons are often made between that of ovarian and that of appendical origin. Nearly all authors have agreed that the former is always cancerous while the latter is not, though it frequently leads to a fatal outcome. The gravity of appendical pseudomyxoma is more likely to be proportional to the degree of involvement. The cause of death of patients with this type has been best explained by Pfannenstiel (cited by Antoine [6]), who spoke of a "function death" (*Funktiontod*): The peritoneum cannot handle the large mass of gelatinous material; it cannot absorb it, and a great part of the peritoneal surface becomes blocked. It then is no longer able to overcome minor insults or insignificant infections. Usually death is not an acute but a slow, exhaustion process.

The various arguments for and against the cancerous nature of pseudomyxoma of appendical origin are based on clinical observations, speculative reasoning and histologic studies.

Hudacek [7] is convinced that the condition is not cancerous because cachexia and recurrences following operation have never been recorded. The experimental production of mucocele and pseudomyxoma in the rabbit by Naeslund was cited by Hudacek as additional proof that the condition is not cancerous. Antoine [6] did not agree that pseudomyxoma of ovarian origin is caused by transplanted cancer cells while that of appendical origin is due to implantation of normal cells. The fact that the mucosal epithelial cell changes its normal characteristics and suddenly begins to produce gelatinous material, which it never produces under physiologic conditions, marks it as a transformed cell, the transformation having taken place within the mucocele as a result of the atresia. Once its nature has changed, the cell will continue to produce gelatinous material even when it is thrown out of the atretic cavity onto the peritoneal surface. Antoine [6] opposed the view that the gelatinous material is condensed mucus, due to absorption of water, because the increased intraluminal pressure which was responsible for the absorption is no longer present in the peritoneal cavity.

A somewhat similar view was expressed by Woodruff and McDonald [8] on histologic grounds. These authors stated that simple mucoceles hardly ever rupture and that even when their contents have been discharged into the peritoneal cavity a frank picture of pseudomyxoma does not result. On the other hand, a small proportion of mucoceles (about 7 per cent of their series) show adenocarcinoma arising within the lumen; these mucoceles generally rupture and disseminate cancer

5. Hentz, V. G.: Ann. Surg. **96**:456, 1932.
6. Antoine, T.: Ztschr. f. Geburtsh. u. Gynäk. **111**:37, 1935.
7. Hudacek, E.: Arch. f. klin. Chir. **155**:694, 1929.
8. Woodruff, R., and McDonald, J.: Surg., Gynec. & Obst. **71**:750, 1940.

cells, bringing about fatal pseudomyxoma. These authors propose the hypothesis "that the Grade I adeno-carcinoma in appendiceal cysts is the result of malignant change which takes place somewhere in the pathogenic cycle of the simple mucocele and that this in turn is the result of an obstructive process in the lumen of the appendix."

D'Aunoy and Fine,[9] presenting the results of their own histologic studies and also expressing Lejar's views, concluded that pseudomyxoma in general (ovarian or intestinal) should be classed as adenocystoma for the reason that epithelial cells usually line the invaginations caused by the mucous deposits on the peritoneal surfaces. Whether the cystadenoma is cancerous or not depends on the nature of the cells at their source of origin.

It is often difficult to establish on histologic grounds whether pseudomyxoma is cancerous or not, even when the original focus is known to have been cancerous. This is true of mucoid carcinoma in general. McCrae and Coplin [10] attributed this difficulty to an epitheliolytic agent of humoral origin which obscures the morphologic characteristics of the cancer cells.

A more conservative line of reasoning has been expressed by some authors regarding the cytologic findings in pseudomyxoma of appendical origin. Trotter [11] was in favor of viewing pseudomyxoma as a "foreign body peritonitis," and he cited Nager, who aptly used the term "cystic endothelioma" to describe pseudomyxoma of appendical origin. According to Trotter:

> . . . there is a tendency for the peritoneal endothelium to grow over and cover in the foreign substance. On the other hand, the endothelium may persist under cover of a layer of mucinous material, and in this case the cells are apt to become cubical or columnar. Some observers regard these as derived from the epithelial lining of the appendix, others think that they are due to transformation of the peritoneal endothelium (a more likely view, if we bear in mind the capacity of these cells to show cubical or columnar cells under abnormal circumstances).

That one must exercise great caution in interpreting morphologic observations on cells in peritoneal lesions is brought out by the work of von Brunn [12] and that of Cunningham.[13] According to von Brunn, the surface cells of the serosa are of epithelial nature and possess the property of forming cilia or of arranging themselves in tubular formation under special conditions. Cunningham by injecting whole or laked blood into the peritoneal cavity caused the serosal cells to become first cuboid and finally columnar.

On summing up the various views regarding the possible role of the epithelium from the appendical mucosa in the production of peritoneal pseudomyxoma it is apparent that the majority of authors have held the view that dissemination of epithelial cells always follows rupture of a mucocele and that the epithelium-like cells which are frequently observed on eroded peritoneal surfaces had their origin in the appendix. The experimental work of von Brunn and that of Cunningham, however, tend to cast doubt on the correctness of this contention.

At the conclusion of the previous work we gained the impression that the material from the mucoceles might contain living cells which were responsible for its ability to spread and to be transplanted.

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9. D'Aunoy, R., and Fine, A.: Am. J. Cancer **22**:59, 1934.
10. McCrae, T., and Coplin, W. M. L.: Am. J. M. Sc. **151**:475, 1916.
11. Trotter, W.: Brit. M. J. **1**:687, 1910.
12. von Brunn, M.: Beitr. z. path. Anat. u. z. allg. Path. **30**:417, 1901.
13. Cunningham, R. S.: Bull. Johns Hopkins Hosp. **35**:111, 1924.

The present work was undertaken to test the soundness of that impression by subjecting the material to different biologic tests. It was reasoned that living cells would be injured or killed by abnormal temperatures. It was also assumed that the viability of the cells would be impaired if the material was left out of the animal body for various lengths of time. Again, it was planned to inject the material into animals of different species on the assumption that heterologous transplants would not survive in the new host.

FIRST PART OF EXPERIMENTAL WORK

At the outset of this work we undertook first to procure enough material for the various injection and transplantation experiments. The work was begun on June 18, 1941 and on July 17 and 21 we obtained the first supply of material, in rabbits 5 and 6. In each of these animals a large mucocele was produced within eighteen days after ligation.

The supply of material was particularly abundant in rabbit 6, in which the mucocele measured 13 cm. in length, 7 to 8 cm. in diameter in the proximal two thirds and 2.5 cm. in diameter in the distal third. The mucocele in rabbit 5 was considerably smaller (10 by 2.5 cm.) yet large enough to furnish sufficient material for further investigation.

The material from rabbit 5 was very thick, having the consistency of cream cheese. This was emulsified with isotonic solution of sodium chloride and used in the following manner:

1. A portion was injected into the marginal veins of the ears of 2 rabbits.
2. A portion was injected intraperitoneally into 2 rabbits.
3. A portion was heated in a water bath at 50 C. for sixty minutes and then was injected intraperitoneally into 2 rabbits.
4. A portion was left standing at room temperature for thirteen days and then was injected intraperitoneally into 1 rabbit.

The content of the mucocele of rabbit 6 was not nearly as thick and pultaceous as that from the mucocele in rabbit 5. The material had a glycerin-like viscid base intermixed with thick white "curds." It proved to be sterile after seventy-two hours. This was used in the following manner:

1. A portion was injected intraperitoneally into 1 rabbit, 1 guinea pig and 2 rats.
2. A portion was heated in a water bath for thirty minutes at 56 to 60 C. and then was injected intraperitoneally into 1 rabbit, 2 guinea pigs and 2 rats.
3. A portion was left standing at room temperature for eleven days and then was injected intraperitoneally into 1 rabbit and 2 rats.

Similar experiments were done with the material obtained from rabbits 9, 19, 20 and 22.

The results of the various injections were as follows:

1. Intravenous injections: Four rabbits were used. They all remained in good health. Two of these animals were killed in order that we might explore their lungs, spleens, kidneys, etc. None of these animals showed any embolic foci, though the emulsions used for the intravenous injections were fairly thick. (Large rabbits were picked for these injections so that it would be possible to introduce a 20 gage needle into the marginal vein of the ear.)

2. Intraperitoneal injections with unheated material: Of the 7 rabbits used, 4 did not show peritoneal involvement. In the remaining 3 the injected material formed encapsulated nodules attached to peritoneal surfaces. Thin veils of peritoneal adhesions surrounded the nodules. The appearance of the lesions suggested local irritation from the mere presence of the foreign substance.

3. Intraperitoneal injections with heated material: Of the 7 rabbits used, 3 did not show involvement. Two of the remaining 4 had nodules formed by the condensed injected material, attached to the wall of the intestine and to the mesentery, respectively. One rabbit had a necrotic nodule attached to the abdominal wall, and some intestinal loops were adherent to the abdominal wall in this region. One rabbit had two nodules in the greater omentum; one of these was seemingly formed from the condensed injected material, while the other had a thick fibrous capsule and a necrotic core.

4. Intraperitoneal injections in rats: Eight rats were used. No peritoneal involvement was found in any.

5. Intraperitoneal injection in guinea pigs: Of the 5 animals used, 1 showed several small necrotic nodules on the under surface of the diaphragm.

6. Intraperitoneal injections of material which had been standing at room temperature for various lengths of time: Ten rabbits were given intraperitoneal injections of this material. In 6 of them the injections were not followed by any peritoneal involvement. One rabbit had a small seminecrotic area in the wall of the abdomen along the line of passage of the needle. The omentum was adherent to the abdominal wall in this region and contained numerous necrotic nodules. We resected the entire omentum. Two rabbits showed necrotic nodules on the peritoneum, and these contained gram-negative bacilli on culture. One rabbit had several necrotic nodules in the deeper layer of the skin. One rabbit had a small necrotic nodule adhering to two adjacent intestinal loops. The nodule was carefully shelled out and then deposited in the peritoneal cavity of another rabbit; at the same time it was crushed and its white pultaceous contents liberated. The peritoneal cavity of the latter animal was explored five weeks later. Numerous nodules were scattered through the cavity, some of these the size of a pea and some as large as a plum. Each of these nodules had a soft putty-like center. Cultures of the material from these nodules proved to be sterile.

Analysis of the Findings.—Intravenous injections of the material from mucoceles failed to produce lesions in any organs of the rabbits. Rats inoculated intraperitoneally with the material did not show any "tumors." Of the 5 guinea pigs inoculated, 1 presented necrotic nodules. Intraperitoneal injections of heated and unheated materials gave nearly similar results in rabbits. There was no perceptible difference in results from fresh and old materials used for injections. None of the intraperitoneal injections consistently produced secondary growths.

Evaluation of the Findings.—No definite conclusions could be drawn from the findings but the preponderance of evidence was against the view that the cellular elements of mucoceles are transplantable. The most one could claim for the material was that it might act as an irritant, favoring the production of inflammatory lesions. Again, we suspected that two other factors could have been involved in the production of the lesions, namely, the responsiveness of the rabbit's tissues to irritation and microbial agents. The material in mucoceles of short standing frequently contained bacteria. True, the material often became sterile on standing, and the peritoneal deposits which followed rupture of mucoceles were usually sterile, yet the factor of bacterial irritation could not be overlooked as a possible cause for the occasional development of necrotic nodules following intraperitoneal injections.

An accidental discovery confirmed our suspicions. One of the rabbits which we intended to use for ligation of the appendix had been used about two months previously for Friedman's test. The abdomen had been explored at that time. In the present operation we found deep in the scar several small nodules with necrotic centers, similar to those encountered in our inoculation experiments. One of these nodules was crushed and its content deposited in the peritoneal cavity of the same rabbit. The cavity was explored seven weeks later. Numerous "tumors," discrete and confluent, were scattered through it. They varied in size from that of a pea to that of a hen's egg. Each "tumor" had a thick fibrous wall and a white pultaceous core. The material was cultured both aerobically and anaerobically. No growth was obtained in any of the cultures.

These findings marked the end of the first part of this work. Our final conclusion about the material from the mucoceles was: While it may have contained living cells, such cells did not possess any proliferative qualities and were not responsible for the spread of the mucous deposits.

SECOND PART OF EXPERIMENTAL WORK

The second part of this work was devoted to study of appendical mucocele and pseudomyxoma from the standpoint of pathology.

In our previously published work [1] nearly all the mucoceles were of but a few weeks' standing (only 1 rabbit in that series was permitted to go for eight weeks after ligation, while most of the others were examined within fifteen to thirty days). Again, in the first part of the present work, when our main aim was to procure material from mucoceles for injection experiments, the average time between the ligation of the appendix and the exploration was twenty days (the shortest seven days and the longest thirty-six days). In the second part of this study it was decided to permit the animal to carry the mucocele as long as possible and to investigate all the changes in the tissues after the process had run its course.

Eight rabbits were used. Two of these (27 and 29) died of ruptured mucoceles on the twentieth and twenty-fifth days. Incisional hernias developed in 2 rabbits (16 and 35), and the hernias contributed to the death of these animals: In one the increased intraperitoneal pressure created by the large mucocele and secondary deposits forced the abdominal contents into a comparatively small hernial ring and brought about death from strangulation of the intestinal coils on the fifty-fifth day. In the other rabbit a huge ventral hernia developed within about two weeks after ligation of the appendix. The abdominal contents could be felt through the thin skin covering the abdomen. The mucocele in this animal, as established later, was the largest we encountered. The entire hernial sac with its contents was dragging and rubbing against the floor of the cage. This finally led to evisceration of all the peritoneal contents and death of the animal on the fifty-first day.

One rabbit (28) died on the eighty-ninth day. Exploration revealed a thick apron-like necrotic structure covering the stomach, intestines, liver and spleen. The liver and the spleen were enveloped by this structure in a glovelike manner and were literally choked by it.

Two rabbits (25 and 34) had spherical bodies of inspissated mucus within the mucoceles (myxoglobulosis). The peritoneal cavities of these animals were explored on the seventieth and eighty-fourth days, respectively. The globules differed somewhat in appearance; they were nearly transparent and glassy in one animal and opaque and totally white in the other.

One rabbit (8) was kept for four months after ligation of the appendix. This rabbit had been used about eight months previously for inoculation experiments. It was the rabbit which showed numerous necrotic nodules in the greater omentum and from which the entire omentum was resected. At the time of the ligation of the appendix the peritoneal cavity did not show any vestiges of previous involvement. The rabbit was killed one hundred and twenty-one days after ligation, and the mucocele was the second largest obtained.

Macroscopic Observations on Mucocele and Peritoneal Pseudomyxoma.—The largest mucocele in this series (rabbit 35) measured 15 cm. in length, 6 to 7 cm. in diameter in the proximal half and 2.5 cm. in the distal half. This mucocele, however, ruptured when an attempt was made to dissect it from the mass of adhering intestinal coils. The second largest (rabbit 8) measured 13 cm. in length and 4 to 5.5 cm. in diameter; a diverticulum, 3 cm. in diameter, was given off from its wider part (fig. 1 *A*). A physical explanation for the enormous size of each of these mucoceles could be found in the roominess of the abdominal cavities in these 2 animals. In rabbit 35 the separation of the abdominal muscles was complete, and the skin over the abdomen formed the only outer covering. The skin stretched easily and permitted free expansion of the abdominal contents. In rabbit 8 the previous resection of the greater omentum might have been a contributing factor, but aside from that this animal was the oldest and the largest of the entire group.

Most of the other mucoceles were smaller. The enlargement and the stretching of the mucoceles were usually not uniform. The tendency toward ballooning and formation of a diverticulum was a common finding. In many instances the ballooned portion was several times the diameter of the original viscus.

Escape of the mucoid material occurred in a greater or lesser degree in every one of the mucoceles, as evidenced by the various peritoneal lesions; these ranged from slimy adhesions to various mucoid deposits, encysted or free. In some instances the mucocele appeared intact at the time of exploration, but the surrounding adhesions testified to the fact that some leakage had occurred.

Peritonitis of the foreign body type always accompanied the escape of mucus into the cavity. The nature of the exudate, however, varied with the cellular response. This was true not only of the material which had escaped into the peritoneal cavity but also of the material formed within the mucoceles. In its

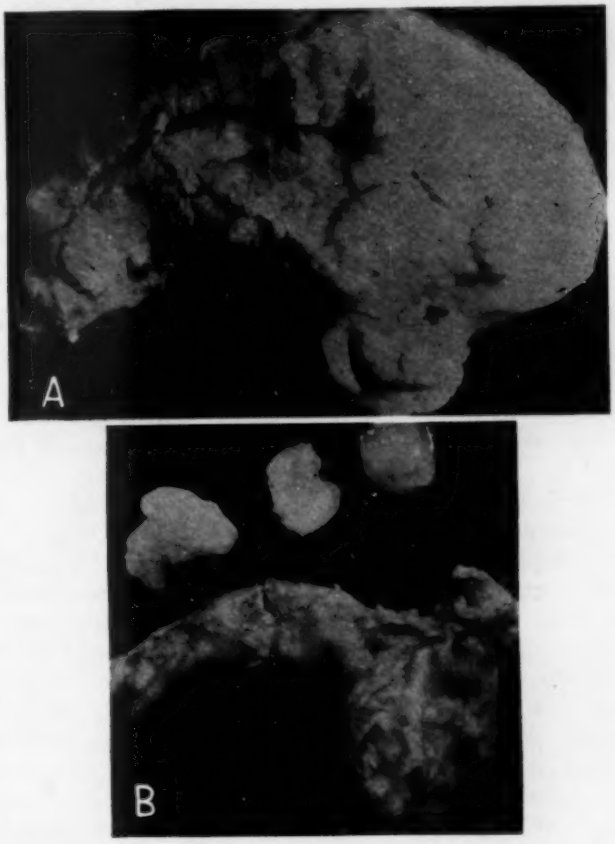

Fig. 1.—*A*, mucocele from rabbit 8. Note the diverticulum formation. *B*, mucocele and some of its globular contents from rabbit 34. The diameter of the diverticulum is nearly three times that of the mucocele proper.

purest form the mucus within the mucoceles had the appearance and consistency of glycerin; i. e., it was translucent, colorless and fairly thin yet highly viscid. However, this type of mucus, without admixtures, was rarely seen. Material resembling milk curds was generally intermixed with the mucus in greater or lesser proportions. The curdlike solid particles consisted of leukocytes and epithelial cells in various stages of disintegration. As the relative content of necrotic cells and debris became greater, the material assumed a white color and became thicker. In extreme stages the material was of the color and consistency of cream cheese. The peritoneal deposits were more apt to be of the white pultaceous variety.

In the 2 cases of myxoglobulosis the marble-like bodies were of about the diameter of the mucocele enclosing them. In one instance the mucocele had a relatively large diverticulum (fig. 1 *B*), but the globules were found in the narrow, nondistended part, while the diverticulum contained thick glistening mucus. In the other case the mucocele was uniformly narrow throughout. The globules were nearly transparent in one case, though even these when cut across had more solid white and opaque particles incorporated in their makeup. The globules in the other case were more homogeneous, white and solid.

Microscopic Observations.—As mentioned in the previously published paper,[1] bacteria could generally be demonstrated in the material from mucoceles by study of hanging drops, stained smears and cultures. They were mainly gram-negative bacilli and occasional heavy gram-positive bacilli. Some of the material evacuated from a large secondary implant was centrifugated and the sediment hardened in 4 per cent solution of formaldehyde. This was embedded in paraffin and sectioned. The sections consisted of leukocytes, large mononuclear cells and broken-down cell remnants. The material from secondary implants always contained leukocytes in various stages of disintegration but no bacteria; cultures of the same were nearly always sterile. Moreover, the material seemingly possessed bacteriostatic properties. We have kept some of it in the refrigerator for about sixteen months. This material was collected in a clean but not sterile jar and was loosely covered. No preservative was added to it, yet no evidence of decomposition could be detected. After the material had stood for over eight months it was cultured on various mediums, but no growth was obtained. However, the addition of this material in various concentrations to cultures of staphylococci failed to inhibit their growth. From these observations we surmise that the material cannot support bacterial life.

In order to evaluate properly the histologic changes which took place in the experimentally produced appendical mucoceles, it will be helpful to go over the structure of the normal rabbit appendix. The appendix of this animal is mainly a lymphoid organ; approximately 75 per cent of the thickness of its wall is made up of lymphoid tissue. Most of the remaining 25 per cent consists of the mucosa, and only 4 to 5 per cent is made up of fibromuscular tissue. Briefly, there is a rim of fibrous tissue on the surface from which numerous thin septums extend toward the center like the spokes of a wheel, subdividing the bulk of the lymphatic tissue into conelike nodules. The mucosa with its tubules penetrates part way between the apexes of the cones in a fringelike manner. The appendix could best be described as consisting of three layers: (1) inner glandular, (2) middle lymphoid and (3) outer fibromuscular. The muscle tissue, however, is so little developed that it can hardly be distinguished from the fibrous serosa; even with special stains (Mallory) difficulty is experienced in separating the two. The lymphatic cones rest on the outer fibromuscular rim, the long axes of the cones running perpendicularly to the long axis of the appendix. It is evident that the appendix is not made to withstand much strain. The mass of lymphoid tissue could not withstand much pressure; the same may be said about the mucosa. The only layer that would offer any real resistance is the outer fibromuscular layer; it, however, is so thin that one would expect it to give way with the slightest increase in pressure. It is likely that the ease with which pseudomyxoma is produced in the rabbit is due in a major degree to the weakness of the appendical wall.

In contrast, the human appendix is built to withstand pressure more effectively. The combined thickness of the submucosa, muscle layers and serosa (fibromuscular

elements) is about 50 to 60 per cent of that of the entire wall. Pressure alone, without superimposed infection, will hardly suffice to produce a break in such a wall.

The histologic changes observed in experimental mucoceles varied, though they followed definite patterns. The foremost change was the thickening of the outer, fibromuscular coat. The thickness of this coat reached 30 to 40 per cent of that of the entire wall, compared with 4 to 5 per cent in the normal appendix. No difficulty was experienced in identifying muscle fibers. The next important change

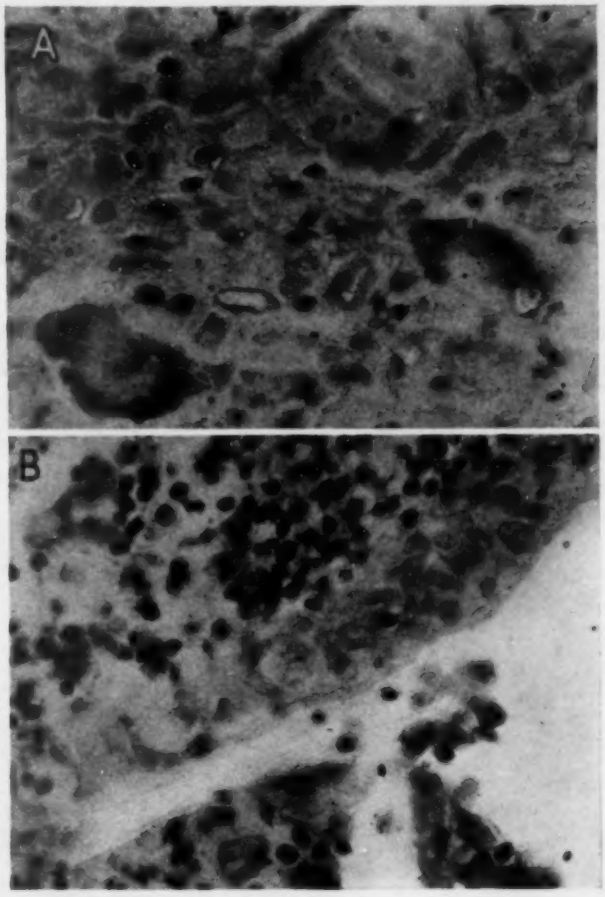

Fig. 2.—*A*, wall of a cystic cavity in a mesentery, showing abundance of giant cells; × 590. *B*, necrotic cavity of an implant, lined by columnar epithelial cells; × 590.

was the thinning out of the lymphoid coat; the process was not only relative, due to the stretching of the entire wall, but also absolute. The lymphatic tissue changed its shape in nearly all the mucoceles and appeared as lenslike oval follicles with the long axes running parallel to that of the outer wall. In most of the mucoceles the thickness of the lymphoid coat was reduced from 75 per cent to about 30 per cent or less, and in isolated instances it disappeared entirely. The mucosa was the least changed; the secreting epithelium appeared to be active, and the distribution of the tubules was little altered in most instances. However, in many

sections taken through the most stretched part of the mucocele there was almost complete atrophy of the mucous glands as well as of the lymphoid tissue.

The characteristic pathologic changes produced in the appendix as well as in the peritoneal implants were of an inflammatory nature and were nearly always accompanied by necrosis. The average sections of the wall of a mucocele contained a mass of degenerated leukocytes and cellular debris adhering to the inner

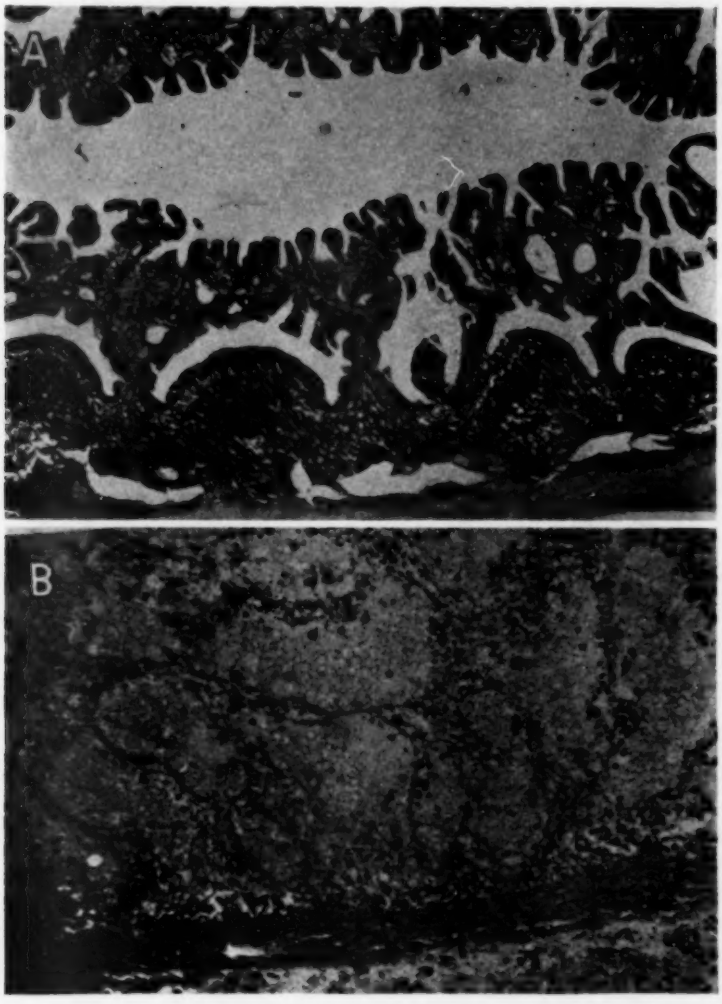

Fig. 3.—*A*, wall of a mucocele in which myxoglobulosis was observed (rabbit 25); × 42. *B*, hemangiomatous appearance of the spleen in rabbit 28; × 140.

surface and penetrating into the wall for various depths, destroying and replacing the normal structures. In some sections tonguelike projections of the necrotic substance could be seen extending through the mucosa and lymphoid structures, to be finally arrested in their progress by the thickened fibromuscular outer coat or to form cystic spaces in that coat. In one section, taken through the appendix and the adjacent mesentery of the small intestine adhering to it, the material could

be followed through the entire wall of the appendix as it extended into a cavity formed in the mesentery. In the zone adjacent to the necrotic deposits could be seen numerous eosinophilic leukocytes, occasional nests of lymphocytes, a sprinkling of plasma cells, occasional large histiocytic cells and foreign body giant cells. Young connective tissue, often myxomatous in character, spread out peripherally to this zone.

All the secondary implants had common histologic characteristics, differing only in degree. The larger type had a central necrotic mass surrounded by a cellular zone of the same description as the subnecrotic zone of the wall of the mucocele.

The walls about the cystic cavities in the mesentery had a similar area of central necrosis, intensely infiltrated by eosinophils and monocytes and variously shaped histiocytic cells intermingled with fat cells. Giant cells were abundant in places (fig. 2 *A*).

Here and there through the nodular implants as well as through the walls of the mesenteric cavities were seen fragmentary tubular structures lined by epithelial cells; some of these were hard to distinguish from giant cells. It was difficult to determine whether many of the cells were epithelial or mesenchymal in nature. Occasional groups of secreting columnar cells, distinctly epithelial, lined a necrotic cavity (fig. 2 *B*).

Of great interest were the sections of the mucoceles which contained the marble-like bodies of inspissated mucus. As mentioned in the description of gross features, the caliber of these mucoceles in the areas containing the globules was not materially increased over that of the normal rabbit appendix, yet these mucoceles showed decided histologic changes. Sections taken through the parts of the wall containing the globules were nearly of an identical histologic pattern in both mucoceles. They showed marked hyperplastic changes in the mucosa and gland tubules branching out in typical papillomatous fashion, assuming a neoplastic appearance (fig. 3 *A*). Of all the mucoceles, these were the only ones that showed such striking similarity to each other and such uniformity and symmetry in the mucosal hyperplasia.

Of interest also were the structural changes produced in the liver and the spleen of rabbit 28. In this animal, as recorded in the description of the gross details, there was an apron-like thick necrotic covering over all the peritoneal contents; this spread out over the liver and the spleen like a bark, nearly enveloping these organs. The envelopment of the spleen was almost complete and the organ was "choked" by the encapsulation. Grossly the splenic tissue was semifluid and almost black. Microscopically the normal landmarks were entirely gone and the tissue consisted of lakelets of blood enclosed within thin-walled cavities. The appearance was suggestive of hemangiomatous transformation (fig. 3 *B*). The changes in the liver were not nearly so conspicuous. They consisted of parenchymal necrosis and hyperplasia of the ducts.

COMMENT

Encouraged by some of the findings recorded in the previous paper,[1] we aimed to prove in the first part of this work that the material from mucoceles carried a potent substance, possibly viable cells, which when implanted in other animals would initiate the formation of "tumors." As the work progressed we became more and more convinced that some of our apparent previous successful results could not be ascribed to potent cells. The reaction of the rabbit's tissue to irritants seemingly was responsible in a great measure for some of the "implants."

On the whole this part of the work did not substantiate the contention that epithelial cells from a ruptured mucocele were responsible for the production of pseudomyxoma. Incidentally, this work may save future authors from basing their conclusions about the proliferative qualities of epithelial cells that have escaped from ruptured mucoceles on our previous experimental findings; this has already occurred in an article by Chaffee and Le Grand.[14]

The second part of this work was given to a study of mucocele and pseudomyxoma from the standpoint of pathology. This study was based in part on the material from the previous work.

We are convinced that the experimentally produced pseudomyxoma is analogous to its clinical counterpart. Occasional minor differences may exist, but these can be readily explained by the anatomic variation of the appendix in man and in the rabbit: Whereas in man this organ has a tough fibromuscular wall, able to withstand great pressure, in the rabbit the amount of fibromuscular tissue is relatively insignificant. For this reason there is in the human appendix a greater tendency toward cystic formation. The fact that pseudomyxoma followed almost every mucocele in our experiments, while in man it is a rare complication, can be readily explained on the anatomic points of difference in the two species.

The microscopic changes in the secondary implants seem to be identical with those in man. The histologic descriptions of Trotter and particularly of D'Aunoy and Fine correspond to our observations even in the smallest details.

The production of myxoglobulosis is not within the control of the experimenter, nor can it be predicted. Among 24 mucoceles (10 in the 1940 group and 14 in this series), we have encountered only 2 in which myxoglobulosis had occurred. Probably a combination of physical conditions is required to bring about the globular formation of inspissated mucus.

Various explanations have been given for this phenomenon. Meyer and Rockstroh[15] expressed the opinion that the more forceful peristalsis from the acutely hypertrophied musculature plus the changing internal pressure may set the mucus into motion and lead to the formation of spherical bodies. These authors cited the opinions of Kaufmann and of Aschoff. According to the former, the globules are formed in blocked glands of the mucosa (like retention cysts) and finally are extruded in their spherical forms. Aschoff suggested that as a result of inflammation the shed cells in the mucus become the nuclei for the formation of the spheres, analogous to the evolution of gallstones.

The histologic appearance in our 2 cases of myxoglobulosis was unique in two respects: (1) The symmetric glandular hyperplasia with the papillomatous mucosa singled the two mucoceles out as belonging to a different entity, and (2) in their identical changes both formed a striking contrast to the rest of the mucoceles, among which no two histologic pictures were alike. It is possible that the similarity of these two mucoceles was coincidental. No histologic constancy or even frequently recurring similarity had been noted in the reported clinical cases. Milliken and Poindexter,[16] for example, gave the following microscopic description of myxoglobulosis: "The wall of the appendix is greatly thickened, the chief constituent being fibrous tissue which in some places has become hyalinized. Very little remains of the mucosa." The same authors cited the microscopic descriptions published by Shattock and Morrison. In the mucocele observed by the former

14. Chaffee, J. S., and Le Grand, R. H.: Arch. Surg. **45**:55, 1942.

15. Meyer, R., and Rockstroh, H.: Ztschr. f. Geburtsh. u. Gynäk. **112**:125, 1936.

16. Milliken, G., and Poindexter, C. A.: Am. J. Path. **1**:397, 1925.

there was evidence of hyperactivity of the mucosal epithelium and destruction of the crypts, while the one observed by the latter showed atrophic changes in the mucosa with disappearance of the epithelial elements.

From the histologic standpoint our 2 mucoceles with myxoglobulosis might fall into the "malignant group" of Woodruff and McDonald. These authors argued that all mucoceles can be divided into two groups: (1) those which contain adenocarcinoma and are responsible for the production of pseudomyxoma and (2) simple mucoceles. Their description of the cancerous group reads as follows: "Regions of final papilla-like meshwork were found in the cysts. This, in contrast to the benign mucoceles, which uniformly showed a smooth lining. . . . Papillary arrangement of the mucous membrane, a comparative hypertrophy of the cells and nuclei, and hyperchromatic nuclei were found in the malignant group."

Woodruff and McDonald are the most outspoken proponents of the view that cancerous changes might take place in a mucocele as a result of obstruction in the lumen and that in patients in whom pseudomyxoma progressed to a fatal outcome it quite likely originated in adenocarcinoma of the appendix. Many other authors view pseudomyxoma as cancerous, though none of these to our knowledge bases his assumption on histologic grounds. Curiously, the very same experimental findings of Naeslund (peritoneal implants resulting from rupture of mucocoles in rabbits) are interpreted differently by different authors. Michaelson, who favored the view that seeded epithelial cells carried by the escaped mucus possess proliferative tendencies which may lead to cancer, mentioned Naeslund's experiments as confirming this view. Similarly, Hudacek, basing his opinion, in part at least on the experimental findings of Naeslund, concluded that pseudomyxoma of appendical origin is always noncancerous.

Evaluating our own findings, we are of the opinion that the various tumor-like implants in the peritoneal cavity which follow rupture of a mucocele are inflammatory in nature. We believe that *the histologic elements which when combined produce the picture of pseudomyxoma are derived primarily from the material from a ruptured mucocele (which may or may not contain mucosal cells) and secondarily, also principally, from the peritoneum and its blood supply, which participate jointly in the production of the inflammatory exudate.* The conception that the disseminated mucoid material is a product of epithelial cells which took on new life after they had lodged in the peritoneal cavity does not impress us as sound. Evidence substantiating that conception to our knowledge is so far lacking. We are inclined to side with the more conservative authors who view the epithelium-like cells as transformed endothelium.

The clinical course of the experimentally produced pseudomyxoma paralleled in a measure the pathologic changes which were taking place in the abdomen of the animal. Some of the animals died soon after rupture of their mucoceles, presumably from an overwhelming toxic reaction. Most of the animals, however, tolerated the inflammatory process fairly well, though with nearly every one there was a period when it appeared listless, did not take its food and was losing weight. Following this period, the animal generally recuperated, though ultimately it died of complications (or was near the point of death when the peritoneal cavity was explored). Mechanical interference with the passage of the intestinal contents was outstanding; the mucocele often occupied the greater part of the cavity, with the intestinal coils flattened against it in a crisscross manner, so that there was difficulty in following the course of an involved segment of bowel. Again, in some instances the necrotic masses of the organized exudate enveloped and compressed entire organs, often obstructing the circulation.

SUMMARY

Peritoneal pseudomyxoma is an inflammatory lesion. It is presumably not bacterial, though one cannot be certain that some bacteria may not have survived within the atretic cavity of the mucocele at the time of its rupture.

The peritoneal deposits and the secondary implants consist principally of an exudate from the peritoneum; the mucoid component in these may be relatively small.

Death as it was observed in rabbits, could be attributed to three causes: (*a*) The mucocele ruptured suddenly and the evacuated contents produced an intense toxic reaction. (*b*) Continued slow leakage produced extensive adhesions terminating in intestinal obstruction. (*c*) The exudate formed a heavy deposit around whole organs eventuating in total arrest of the function of such organs.

In 2 of 24 experimentally produced mucoceles, myxoglobulosis was discovered. The histologic changes in the two appendixes were of a definite pattern and came closer to being neoplastic than those in any of the other sectioned material. The neoplasia, however, was of a type which the pathologist considers inflammatory in nature, namely, that of papillomatosis. Myxoglobulosis is an accidental phenomenon in the evolution of mucoceles, quite likely depending on a combination of physical conditions. In the 2 mucoceles aforementioned the lumen at the site of the globular formation was narrow and the mucosa unusually hyperplastic.

DISSEMINATED LUPUS ERYTHEMATOSUS—AN ALLERGIC DISEASE?

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The clinical and pathologic alterations of disseminated lupus erythematosus present many features which suggest that the disease is primarily a manifestation of hypersensitivity or allergy. Periarteritis nodosa, the pathologic lesions of which resemble those found in certain cases of disseminated lupus erythematosus, must, in view of the recent observations of Rich [1] and Rich and Gregory,[2] be considered to have a fairly well established allergic basis. These investigators have shown that the offending antigen in periarteritis nodosa may be a foreign protein or some compound formed by conjugation with the sulfonamide drugs. Presumably other antigens, as yet unsuspected, may be involved. As a result of this work there is a strong tendency to regard periarteritis nodosa as a type of reaction pattern, rather than as a disease with a single etiologic basis.

From time to time it has been intimated that disseminated lupus erythematosus might have an allergic background, but definite evidence for this concept is lacking. With the exception of actinic rays, which have been suspected as the sensitizing agent in certain cases,[3] evidence pointing toward a specific antigen has not been presented. For this reason it seems pertinent to present a case in which there is presumptive evidence that a foreign protein (horse serum) was the irritating factor and perhaps the actual cause of a disease process clinically and pathologically typical of disseminated lupus erythematosus.

REPORT OF A CASE

C. P., a white girl of 17 years, a nursemaid, was in good health until six weeks before admission to the hospital. At that time she accidentally sat on a crocheting needle which pierced the skin of the buttocks. She was taken to the St. Louis City Hospital immediately, where the wound was sutured and a prophylactic dose of antitetanic serum was given. She was permitted to return home.

The wound healed without infection, and except for moderate discomfort related to the location of the injury there were no symptoms. Approximately one week later, however, a "rash" appeared on the face, involving the forehead, the nose and the cheeks. The "rash" was itchy, reddened and slightly raised. The appearance of the facial eruption was accompanied by swelling of the ankles and of the interphalangeal joints of both hands. She returned to the St. Louis City Hospital, where she was told that her symptoms were due to "serum sickness" and was sent home again.

During the subsequent four weeks the patient "never felt quite well again." Although the swelling of the joints gradually disappeared, the "rash" persisted. In the next two week period "sore throat and diarrhea" developed. These symptoms were followed by soreness and bleeding of the gums. In the two day period before hospitalization she had several small "nosebleeds."

The past history was not significant. She had had the usual childhood diseases without complications.

Examination on admission showed a moderately well developed white girl of about 17 years, who was in no acute distress. She appeared nervous and talked constantly throughout

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From the Department of Pathology, St. Louis University School of Medicine.

1. Rich, A. R.: Bull. Johns Hopkins Hosp. **71**:123, 1942.

2. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **72**:65, 1943.

3. Ludy, J. B., and Corson, E. F.: Arch. Dermat. & Syph. **37**:403, 1938. Banks, B. M.: New England J. Med. **225**:433, 1941.

the examination. Her hands were never still, and she picked at her face frequently. A slightly raised erythematous eruption was observed involving the skin of the forehead, cheeks and bridge of the nose. The eyes and the ears appeared to be normal. The nares contained clotted blood. The tongue was "furred" and reddened along the edges. The gums were also reddened and partly covered by dried blood clots. The pharynx was inflamed. The heart was not enlarged to percussion, and there were no thrills or murmurs. The lungs were clear. The blood pressure was 120 systolic and 70 diastolic. The temperature was 101 F., the pulse rate was 100 and the respirations were 20 per minute. The abdomen was not distended, and there were no palpable masses. The reflexes were considered hypoactive. The superficial lymph nodes were not enlarged.

During her stay in the hospital of approximately three months her temperature ranged between 101 and 105.6 F. The latter rise was associated with physical and roentgenographic findings of "pneumonia" at the upper lobe of the left lung. Three weeks after admission "the facial eruption was more pronounced," and two weeks later the cheeks were described as "more reddened by macular areas." Vitamin C saturation tests on the urine indicated definite deficiency. Large doses of that vitamin accompanied by "liver" (preparation not specified) failed to control the bleeding from the gums.

Repeated examinations of the peripheral blood revealed moderately severe secondary anemia. The leukocytes numbered 5,400 per cubic millimeter three days after admission and rose to 18,400 shortly before death. The blood platelet count was 160,000 per cubic millimeter on admission and 190,000 one week later. Aside from slight albuminuria and occasional "pus cells," repeated urinalyses gave negative results. The bleeding and the clotting time were thirty seconds and three minutes, respectively. The Kahn test and repeated cultures of the blood gave negative results.

Three days before death a pericardial friction rub was discovered, and a series of roentgenograms demonstrated an increasing cardiac shadow. Pericardial aspiration was considered ill advised because of the patient's moribund condition. Sulfathiazole was given for several days without beneficial effect. The day before death the abdomen became distended and there was "shifting dulness in the flanks."

Necropsy Report.—At necropsy there were bilateral pleural effusion and marked hydroperitoneum. The pericardium was distended with about 500 cc. of serofibrinous exudate; both the visceral and parietal surfaces were covered by a thick layer of fibrin, giving them a bread and butter appearance. The heart was enlarged, weighing 385 Gm. On section all four valves were distorted by vegetations of varying size and shape. Those on the mitral, tricuspid and pulmonic valves were large, measuring up to 1 cm. in the largest diameter. A single vegetation on the aortic valve was 0.5 cm. in the maximum diameter. All the vegetations were similar. They were yellow-white and not very friable. They were not limited to the "line of closure" of the valves but extended to the free border. Those on the semilunar valves were situated at the junction of the commissures. In some areas the vegetations extended to the chordae tendineae and along the mural endocardium of the auricles and ventricles. Bacteriologic examination of the vegetations gave "negative" results. It was felt that the cardiac lesions conformed to those originally described by Libman and Sacks [4] as an atypical form of verrucous endocarditis, a condition which is now considered to be part of the syndrome of disseminated lupus erythematosus.[5]

The lungs showed serofibrinous pleuritis and bronchopneumonic foci. The liver was slightly enlarged and marked by numerous tiny yellow areas which microscopically proved to be foci of necrosis centering around the efferent veins. The spleen weighed 220 Gm. and was moderately firm. The pancreas, the adrenal glands, the intestines and the genital organs were essentially normal. The lymph nodes were not enlarged.[6]

The kidneys were somewhat swollen and rather soft, with smooth, pale brown surfaces. On section, the cut surface was pale, and the cortex was not diminished in thickness. Microscopically, nearly all the glomeruli showed eosinophilic foci involving part or all of the glomerular tufts, suggesting an acute necrotizing process. In many glomeruli the tufts were thickened so that the capillaries stood out prominently, resembling the "wire loops" described by Baehr, Klemperer and Schifrin [7] in disseminated lupus erythematosus.

4. Libman, E., and Sacks, B.: Arch. Int. Med. **33**:701, 1924.

5. Belote, G. H., and Ratner, H. S. V.: Arch. Dermat. & Syph. **33**:642, 1936. Denzer, B. S., and Blumenthal, S.: Am. J. Dis. Child. **53**:525, 1937. Keil, H.: Arch. Dermat. & Syph. **34**:124, 1936.

6. Fox, R. A., and Rosahn, P. D.: Am. J. Path. **19**:73, 1943.

7. Baehr, G.; Klemperer, P., and Schifrin, A.: Tr. A. Am. Physicians **50**:139, 1935.

The anatomic diagnosis was disseminated lupus erythematosus, atypical verrucous endocarditis involving all four valves, hypertrophy of the heart, acute fibrinous pericarditis and pleuritis, ascites, bilateral hydrothorax, hydropericardium, focal necrosis of the liver and lobular pneumonia.

COMMENT

Klemperer, Pollack and Baehr [8] recently characterized disseminated lupus erythematosus as a "diffuse collagen disease." They reviewed the histologic observations in 20 of their most recent cases and concluded that the manifold alterations of the disease process are localized primarily in the collagenous tissues of the body. They pointed out that "the characteristic organic changes, previously considered as heterogeneous, can now be understood as local manifestations of the widespread damage of collagen."

This concept is a definite advance in the comprehension of disseminated lupus erythematosus in that heretofore, guided by the writings of Baehr, Klemperer and Schifrin [7] and of Gross,[9] attention was focused on the vascular aspects of the disease. Klemperer and his co-workers pointed out that the widespread vascular damage is evident by virtue of a basic injury primarily localized in the connective tissues of the blood vessels and not in the endothelium as believed by Gross. They contend that the basic alteration common to all lesions observed in disseminated lupus erythematosus, whether in the endocardium, pericardium, glomeruli, spleen or blood vessels, is "fibrinoid degeneration of collagen." A process essentially similar but with varied anatomic distribution is believed to occur in the lesions of scleroderma,[10] rheumatic fever, periarteritis nodosa and thromboangiitis obliterans.[11]

Klemperer and his associates were aware of the fact that fibrinoid degeneration had been described as an expression of a hypertensive state.[12] Gerlach [13] described it in his work on the Arthus phenomenon and considered it "an essential feature of hyperergic inflammation." In comparing the pathologic-morphologic observations in disseminated lupus erythematosus and periarteritis nodosa, Klemperer, Pollack and Baehr found that "while fibrinoid degeneration of collagen is common to both diseases, in the latter it is restricted to the vascular system." Furthermore, they stated that "the classical evidences of hypersensitivity so frequently observed in periarteritis nodosa are lacking in disseminated lupus erythematosus."

Any one who is familiar with the clinical course of disseminated lupus erythematosus cannot help taking exception to this statement. The disease has so many features in common with hypersensitive states and particularly with serum sickness that one gains the impression that disseminated lupus erythematosus could be a manifestation of prolonged "serum sickness."

According to Mackenzie,[14] serum sickness is characterized by cutaneous manifestations of an urticarial or an erythematous nature, which usually involve the face and frequently appear over the chest, the back or the extremities. Purpuric rashes of more than average severity are occasionally observed. Fever and edema both occur in about one third of the cases. Moderate oliguria, albuminuria and

8. Klemperer, P.; Pollack, A. D., and Baehr, G.: J. A. M. A. **119**:331, 1942.

9. Gross, L.: Am. J. Path. **16**:375, 1940.

10. Masugi, M., and Yā-Shu: Virchows Arch. f. path. Anat. **302**:39, 1938.

11. Roessle, R.: Klin. Wchnschr. **15**:809, 1936. Jäger, E.: Virchows Arch. f. path. Anat. **284**:526, 1932.

12. Klemperer, P.; Pollack, A. D., and Baehr, G.: Arch. Path. **32**:569, 1941.

13. Gerlach, W.: Virchows Arch. f. path. Anat. **247**:294, 1923.

14. Mackenzie, G. M., and Hanger, F. W.: J. A. M. A. **94**:260, 1930.

cylindruria are not unusual concomitants of the edema. Von Pirquet and Schick [15] found that children show leukocytosis during the prodromal period of serum sickness and leukopenia after symptoms develop. Slight or moderate enlargement of the superficial lymph nodes is usually present. From 50 to 60 per cent of patients with serum sickness show some evidence of arthritis. In summary, then, serum sickness includes cutaneous manifestations, fever, lymphadenopathy, edema, arthritis, leukopenia, purpura and disturbances of renal function.

Certainly all these findings have been observed in cases of disseminated lupus erythematosus. Even involvement of the endocardium is not entirely foreign to all patients suffering from serum sickness. Clark and Kaplan [16] described endocarditis, arterial and other mesenchymal alterations associated with serum sickness in 2 fatal cases. That the particular lesions—for example, the cutaneous manifestations or the extent of the renal damage or the endocardial involvement—may differ in the two conditions is not questioned. However, it is difficult to deny the presence of similar symptom patterns in the two syndromes; the principal difference rests in the fact that one condition (serum sickness) is a disease of short duration which only infrequently terminates fatally, while the other (disseminated lupus erythematosus) is a protracted disease to which the patient almost invariably succumbs. This difference, as well as the differences which are found in the specific lesions, however, suggests a variation of degree rather than of kind. It is as if on the one hand (serum sickness) there were a single application of a sensitizing agent to which the patient reacts in a specific manner and from which he recovers once the body mechanism has overcome its effects. If the same or a similar sensitizing agent is reapplied in sufficient dosage at a later date, a similar train of symptoms ensues. On the other hand, in regard to disseminated lupus erythematosus one might conjecture that the patient is reacting to a sensitizing agent which is being applied repeatedly or which is stored in the body and released slowly over a long period. This thought would tend to characterize disseminated lupus erythematosus as a chronic reaction of the "serum sickness" type.

In suggesting such a concept it is important for one to have in mind an agent to which patients suffering from disseminated lupus erythematosus might be sensitive and one which has more or less continuous application. After reviewing a large group of reported cases of disseminated lupus erythematosus one cannot help being impressed by the fact that sunlight appears to play some role in this disease. The number of cases in which the ill effects of exposure to sunlight are mentioned is too large to be considered merely of coincidental importance. The association of sunburn or sunbathing with the initiation or exacerbation of symptoms was noted time and again by numerous observers. Bechet [17] in a report on the effects of solar and phototherapeutic irradiation included disseminated lupus erythematosus among those diseases which are affected adversely. Ludy and Corson [3] showed that there were abnormal quantities of hematoporphyrin in the urine of 7 of a group of 15 patients with disseminated lupus erythematosus, a manifestation which led them "to believe that the light factor and the presence of this photosensitizing agent in the blood might account for the production of the cutaneous lesions." In 3 of their patients on whom autopsy was performed hematoporphyrinuria was found before death. In addition, each had "a clear history and a distribution of the eruption indicating exposure to light as one of the factors responsible for the outbreak."

15. von Pirquet, C. F., and Schick, B.: Die Serumkrankheiten, Leipzig, F. Deuticke, 1905.
16. Clark, E., and Kaplan, B. I.: Arch. Path. **24**:458, 1937.
17. Bechet, P. E.: Arch. Dermat. & Syph. **29**:221, 1934.

The effect of continued exposure to ultraviolet rays on laboratory animals in relation to states of hypersensitivity is being investigated in this laboratory at the present time.

That the case reported here falls into the category of disseminated lupus erythematosus cannot be disputed. The clinical picture, which conforms to the general pattern displayed by patients suffering from this malady, is no less typical than the anatomic findings demonstrated at the postmortem examination. The unusual features were the circumstances surrounding the onset of symptoms. A review of the literature failed to disclose a similar case in which the initial symptoms of disseminated lupus erythematosus followed within a week or two the parenteral administration of a foreign protein or conformed so well to the symptom pattern of serum sickness. It may, of course, be argued that the initial episode in this case represented a serum reaction which was coincidental and unrelated to the onset of the disseminated lupus erythematosus, or that the symptoms were those of disseminated lupus erythematosus and not serum sickness from the onset, the antitetanic serum having had no ill effects. A third possibility, however, must be considered, namely, that the foreign protein injected was the sensitizing agent which gave rise to the syndrome of disseminated lupus erythematosus, the initial symptoms of which were indistinguishable from those of serum sickness. Because the onset of the illness occurred approximately seven days after the administration of the antitoxin and because the disease was apparently a progressive one from that time until the death of the patient four months later, the latter possibility is considered the most probable. The fact that thousands of patients with serum sickness recover without apparent residual damage is fully realized, but the occurrence of fatal periarteritis nodosa in the exceptional case [1] is undoubtedly a possibility. A consideration of the case reported here suggests that disseminated lupus erythematosus, like periarteritis nodosa, may represent an uncommon specific type of allergic reaction to a foreign protein antigen in a sensitive person. Again, as in periarteritis nodosa, a careful analysis of a group of cases with the possibility of specific antigens in mind might bring to light information of considerable importance. Rich has already shown that at least two antigens may be involved in periarteritis nodosa. It is equally possible that a variety of antigens, one of which is related to actinic rays, or their effects may be initiating agents in disseminated lupus erythematosus.

SUMMARY

A case is described in which there is presumptive evidence of a relationship between the parenteral administration of a foreign protein and the development of disseminated lupus erythematosus. The similarity in general symptomatology between disseminated lupus erythematosus and serum sickness is discussed. The possibility is suggested that disseminated lupus erythematosus may, like periarteritis nodosa, eventually prove to be a pattern of reaction to a variety of antigens in hypersensitive persons. That one such antigen may be related to the action of actinic rays on the sensitized body is considered. The importance of studying future cases of disseminated lupus erythematosus in relation to specific antigens is stressed.

Case Reports

HEMANGIOENDOTHELIAL SARCOMA OF THYROID GLAND

MILTON D. BOSSE, M.D., PITTSBURGH

Only two reports of hemangioendothelial tumor of the thyroid gland appear to have been published in North America. Rice [1] reported a case of hemangioendothelioma of the thyroid gland in a 50 year old woman. The diagnosis was made by biopsy, and roentgen therapy was given. More than two years later the patient had no enlargement of the neck or other evidence of recurrence or metastasis. Simon [2] reported a case of hemangioendothelial sarcoma of the thyroid gland in a 58 year old white man. This patient was observed in Berne, Switzerland. Metastases were found at autopsy.

Another case of hemangioendothelial sarcoma of the thyroid gland is herewith presented.

A white man 68 years old was admitted to the Western Pennsylvania Hospital complaining of cough and hemoptysis of recent development. In addition he had hoarseness, loss of weight and swelling of the right side of the neck. The latter symptoms had been increasing since their onset about five months previously. In the anterior triangle of the right side of the neck was a mass about 8 cm. in diameter. It was movable but was adherent to the skin in one area. The patient's temperature was normal, but there were moist rales throughout the anterior region of the chest. He failed rapidly and died a few hours after admission.

The autopsy (three hours after death) showed that the enlargement of the neck was caused by a tumor measuring 6.5 by 7 by 10 cm. It replaced all of the right lobe of the thyroid gland except a small portion of relatively normal-appearing tissue at the lower pole. The isthmus and the left lobe appeared normal. The tumor was hemorrhagic, was mottled yellow, gray and red and was firm and nodular but with considerable necrosis evident. It was partially adherent to the skin and invaded the muscle and the connective tissue of the right side of the neck. The right common carotid artery was pushed anteriorly and laterally by the tumor, and the upper end of the mass surrounded this vessel. The tumor partially surrounded the larynx posteriorly and displaced the larynx, trachea and esophagus toward the left. The larynx was partially compressed and the laryngeal mucosa was edematous and hemorrhagic throughout, particularly in the region of the right vocal cord. There was extensive bronchopneumonia of the lower lobe of the left lung, as well as congestion and edema of the other lobes of both lungs. In the apex of the right lung, just beneath the pleura was a firm round nodule, 3 cm. in diameter. Its cut surface presented mottled gray, red and yellow tissue, fairly sharply delimited from the surrounding lung parenchyma.

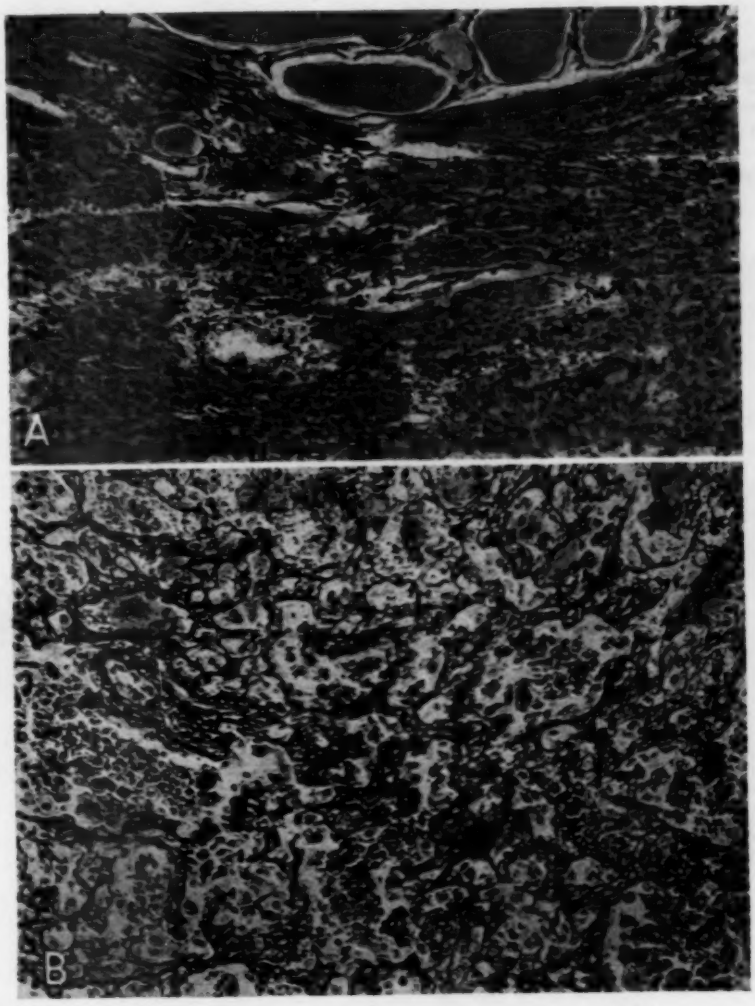
Microscopic examination of hematoxylin-eosin preparations from the tumor of the thyroid gland showed a highly cellular, hemorrhagic tissue. The cells in some areas were compactly and uniformly distributed, and those in other areas were arranged to form small irregular vascular spaces, which frequently contained red blood cells. The cells were quite variable in size and shape; they were more often spindle shaped in the compact areas and somewhat rounded in the areas where they formed vascular spaces. The cells usually had pink cytoplasm, which was frequently vacuolated and often contained phagocytosed red blood cells or brown pigment. Some had clear or foamy cytoplasm. The nuclei were generally rounded but varied greatly in size and shape, and many were hyperchromatic. Mitotic figures were fairly numerous. Some areas contained a number of multinucleated cells. Large areas of hemorrhage and necrosis, as well as areas of fibrosis, were present. The sections from the lower pole of the right lobe showed relatively normal thyroid tissue replaced by the tumor (*A* in figure). In this region scattered acini of the thyroid were mingled with tumor. Sections from the region of the upper part of the larynx showed invasion of the wall by tumor, with extension near the mucosa. Masson's trichrome stain showed moderate amounts of green-stained collagen, while the tumor cells were grayish pink or grayish red. Azocarmine stain showed blue fibers among grayish pink or grayish red tumor cells, and definite blue-stained basement membranes were demonstrable beneath the lining of the vascular spaces. A silver-impregnation preparation showed many reticulum fibers between tumor cells and outlining the vascular spaces

From the Western Pennsylvania Hospital Institute of Pathology (Ralph R. Mellon, M.D., director).

1. Rice, C. O.: Am. J. Cancer **15**:2301, 1931.
2. Simon, M. A.: Arch. Path. **27**:571, 1939.

(*B* in figure). Weigert's elastic tissue stain with superimposed Van Gieson stain showed little elastic tissue in the tumor. Iron stain showed that the brown pigment within the tumor cells contained iron. The nodule in the apex of the right lung was composed of tissue similar in all respects to that of the tumor of the thyroid gland, with formation of vascular spaces, as well as more undifferentiated sarcoma.

The complete anatomic diagnosis was: hemangioendothelial sarcoma of the right lobe of the thyroid gland with extension to adjacent structures and metastasis to the apex of the right lung; extensive bronchopneumonia of the lower lobe of the left lung; fibrous pleural adhesions



A, photomicrograph showing thyroid tissue partially replaced by poorly differentiated tumor tissue; hematoxylin and eosin; × 132.

B, photomicrograph showing the vascular spaces formed by the tumor cells; reticulum stain counterstained with eosin; × 132.

on the right; marked atherosclerosis of the abdominal aorta, and chronic cholecystitis with cholelithiasis.

COMMENT

While it appears that only 2 cases of hemangioendothelial tumor of the thyroid gland have been previously reported in North America, Simon [2] found 20 other cases described in European literature, mostly from Germany and Switzerland.

He stated that 41 cases had been observed from 1907 to 1938 at Bern. Inasmuch as the tumor described by Rice was apparently noncancerous and the one reported by Simon occurred in Switzerland, the present case of hemangioendothelial sarcoma of the thyroid gland appears to be the first reported occurrence of this tumor in North America.

This type of neoplasm of the thyroid gland is of particular interest because Ewing [3] has stated that "the occurrence of true sarcoma in man still requires demonstration."

SUMMARY

A case of hemangioendothelial sarcoma of the thyroid gland in a 68 year old white man is reported. A metastasis was found in the right lung. This appears to be the first reported occurrence of a hemangioendothelial sarcoma of the thyroid gland in North America.

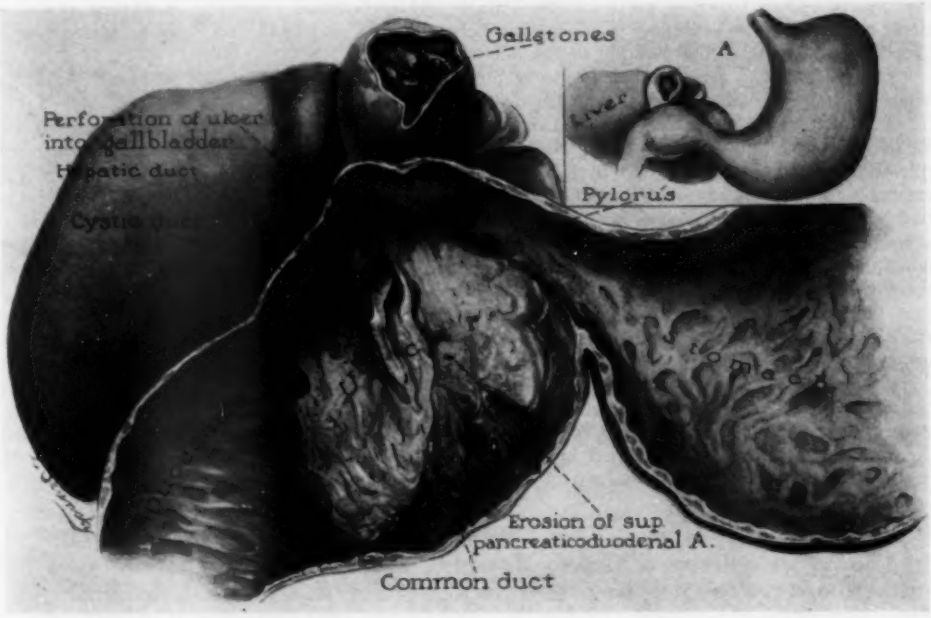
3. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 992.

GIGANTIC NONCANCEROUS DUODENAL ULCER

HAROLD N. GINSBURG, M.D., ELOISE, MICH.

A duodenal ulcer measuring 6.5 to 8.5 cm. in length and 6.0 to 9.0 cm. in width was found in a patient who had suffered from epigastric pain for forty-two years. It had produced pyloric obstruction, had perforated into the gallbladder and had eroded the superior pancreaticoduodenal artery, with fatal hemorrhage. Ulcers of such large size are extremely rare. Only one report of an ulcer of comparable size was encountered in recent American literature, that by Burger and Merrell.[1] Their patient had a duodenal ulcer which measured 2.5 by 5 cm., with symptoms referable to it for thirty-three years.

C. P., a white man aged 70, was last admitted to the William J. Seymour Hospital Jan. 11, 1943 and died the following day.



Artist's drawing showing the large ulcer in the first and second portions of the duodenum, with perforation into the gallbladder and erosion of the superior pancreaticoduodenal artery.

The chief complaints on admission were epigastric discomfort for two weeks and vomiting of red blood for one week. Since 1928 the patient had been admitted to this hospital on numerous occasions, complaining of abdominal pain. Roentgen examination of the gastrointestinal tract in April 1928 showed no abnormality. In September 1935 he gave a thirty-five year history of epigastric pain. The pain would occur two hours after meals and would be relieved by food and sodium bicarbonate. Occasionally he had tarry stools. There was roentgen evidence of diverticulum of the second portion of the duodenum. In September 1939 he was considered to have alcoholic gastritis and arteriosclerotic heart disease. On Jan. 4, 1940 he was admitted complaining of dyspnea, orthopnea, pain in the chest, edema of the ankles, nausea and vomiting. He improved after the administration of digitalis and sedatives. He was again admitted on July 10, when he complained of nausea, vomiting and epigastric pain, and on October 23, when he had complaints of similar abdominal pain, weakness and tarry

From the Department of Pathology (S. E. Gould, M.D., attending pathologist), William J. Seymour Hospital.

1. Burger, G. N., and Merrell, P.: J. A. M. A. **100**:336, 1933.

stools, and was given a Sippy diet, with subsequent improvement. The next admissions were on May 20, 1941, when he had symptoms of pain in the abdomen, vomiting and tarry stools and on Feb. 25, 1942, when he suffered with epigastric and cardiac pain. He was committed to the psychiatric division of the hospital on March 4, as a psychotic senile patient with cerebral arteriosclerosis. On June 23 he again complained of pain in the stomach. At this time the diagnosis was diverticulum of the duodenum, chronic cholecystitis and psychoneurosis.

At the time of his last admission, Jan. 11, 1943, he was pale and acutely ill. The blood pressure was 80 systolic and 35 diastolic. The fundi showed moderate tortuosity of the blood vessels and arteriovenous compression. The lungs were normal. The cardiac rhythm was irregular. There was a rough low-pitched blowing murmur over the apex with its point of maximum intensity 10 cm. to the left of the midsternal line in the fifth intercostal space. The edge of the liver was palpable 2 cm. below the right costal margin, and there was tenderness throughout the abdomen. The extremities were normal.

The urine had a specific gravity of 1.013, a trace of albumin, no sugar and occasional white blood cells. The blood showed a hemoglobin content of 8.3 Gm. (Haden-Hauser), and 2,710,000 erythrocytes and 24,000 white cells per cubic millimeter. Gastric analysis showed 70 per cent hydrochloric acid during fasting and 60 per cent hydrochloric acid after administration of histamine; a test for occult blood disclosed none. Serum albumin amounted to 3.2 Gm., globulin to 2.6 Gm. and nonprotein nitrogen to 75 mg. per hundred cubic centimeters. The electrocardiogram revealed auricular fibrillation and myocardial changes. Roentgen examinations were made on a number of occasions between 1935 and 1942 and showed evidence of a diverticulum in the second portion of the duodenum.

The patient was given a transfusion of 500 cc. of blood. He continued to vomit blood, his blood pressure remained low, and death occurred on Jan. 12, 1943.

At autopsy the stomach was markedly dilated and filled wtih clots of dark red blood. Beginning just beyond the pyloric valve, the duodenum presented a large indurated ulcer, irregularly oval, measuring 6.5 to 8.5 in length and 6.0 to 9.0 cm. in width (figure). The superior and medial edges of the ulcer were sharply depressed for a depth up to 1.0 cm. The left lateral and inferior edges were flat. The base was irregularly nodular and firm, and at a point 5 cm. from the pylorus there was an eroded blood vessel, measuring 3 mm. in diameter, which was elevated several millimeters above the floor of the ulcer. The patent lumen of the vessel measured 2 by 1 mm. The ulcer showed an area of perforation into the gallbladder just beyond the cystic duct. The opening measured 1.2 by 0.3 cm. The gallbladder was thickened and contained twenty-five yellowish brown faceted calculi of uniform size, each measuring about 7 mm. in diameter. The bile ducts were patent.

Microscopic examination of five blocks of tissue from various sites of the ulcer showed chronic ulceration with extensive fibrosis in the duodenum, with areas of epithelial regeneration. There were areas in which the duodenal wall had been completely eroded and in which the floor of the ulcer was formed by the pancreas. There was extensive destruction of the pancreas with fibrosis. No evidence of carcinoma was found.

SUMMARY

The report concerns a man of 70 with a history of peptic ulcer for forty-two years who was found to have an extremely large noncancerous duodenal ulcer. The ulcer produced pyloric obstruction, perforation into the gallbladder and erosion of the superior pancreaticoduodenal artery with fatal hemorrhage.

MULTIPLE MYELOMA WITH LARYNGEAL INVOLVEMENT

BJARNE PEARSON, M.D.; ERNEST STARK, M.D., AND MAXWELL KEPL, M.D.
NEW ORLEANS

Involvement of the larynx in multiple myeloma is rare. Only 2 cases have been recorded in the literature—by Verebely [1] and by Gilmore.[2] Only Verebely's case came to necropsy. Our case is, then, the second case with necropsy to be recorded. In both instances it was demonstrated that the origin of the neoplasm was in a center of ossification in the thyroid cartilage.

A white man aged 54 was admitted to Charity Hospital of Louisiana at New Orleans May 20, 1942 with rheumatic pains and a mass in the left sternoclavicular articulation which had been present for three months. The peripheral blood picture, the blood serum protein and the blood phosphatase were within normal limits. The sternal marrow showed a predominance of pleomorphic cells (60 per cent) with abundant pale blue agranular cytoplasm and eccentrically placed nuclei. A diagnosis of plasma cell myeloma was made.

Roentgen examination showed complete destruction of the medial half of the left and outer third of the right clavicle with a pathologic fracture of the latter. Osteoporosis was seen in two areas of the skull, in the pelvic and lumbar portions of the spinal column, in the middle part of the right ilium and in the sacrum. September 16 a fracture was reported in the middle third of the left humerus. About three weeks prior to the death of the patient hoarseness developed.

The autopsy revealed typical multiple myeloma involving the ribs, the sternum, the clavicles, the right side of the sacrum and the right ilium. Extraskeletal masses were found extending from the sternal ends of the third, fourth and fifth ribs, with invasion into the adjacent muscles, the pleura and the lower lobe of the right lung. There were also extensions of the sacral and ilial lesions into the surrounding tissues. The remaining organs showed nothing of note.

The larynx and trachea were opened by a posterior longitudinal midline incision. Externally and attached to the right half of the cricoid cartilage was an ovoid mass which extended superiorly to and was limited by the arytenoepiglottic fold. It extended laterally and inferiorly, filling the piriform fossa, and penetrated anteriorly the cricothyroid and thyrohyoid muscles. This mass measured cephalocaudad 4.5 cm., laterally 3.5 cm. and anteroposteriorly 2.5 cm. The right vocal cord and surrounding tissues were pushed inward and were slightly infiltrated by the neoplasm, but no fungation was present (figure).

On the anterior aspect of the larynx and trachea, extensions of this tumor were seen to overlie the lower two thirds of the right lamina of the thyroid cartilage and the cricoid cartilage, extending downward to the fourth tracheal ring. The lateral lobe of the thyroid gland on the right had been pushed forward by this mass. The anterior portion of the mass measured 5.5 by 4.5 by 1.5 cm. and extended 1 cm. beyond the midline of the thyroid cartilage.

Serial sections through the tumor and the thyroid and cricoid cartilages were made. In the inferior midportion of the right lamina of the thyroid cartilage a small center of ossification containing marrow, measuring 0.5 by 0.5 cm., was seen. The cartilage at this point was destroyed by the infiltrating tumor tissue.

Microscopic Study.—Sections taken from various portions of the tumor and overlying muscles showed diffuse infiltration by plasma cells in various stages of development (figure). These had invaded the interstices of the muscle fibers. Where this had occurred, the muscle fibers showed loss of staining reactions, cloudy swelling and fragmentation.

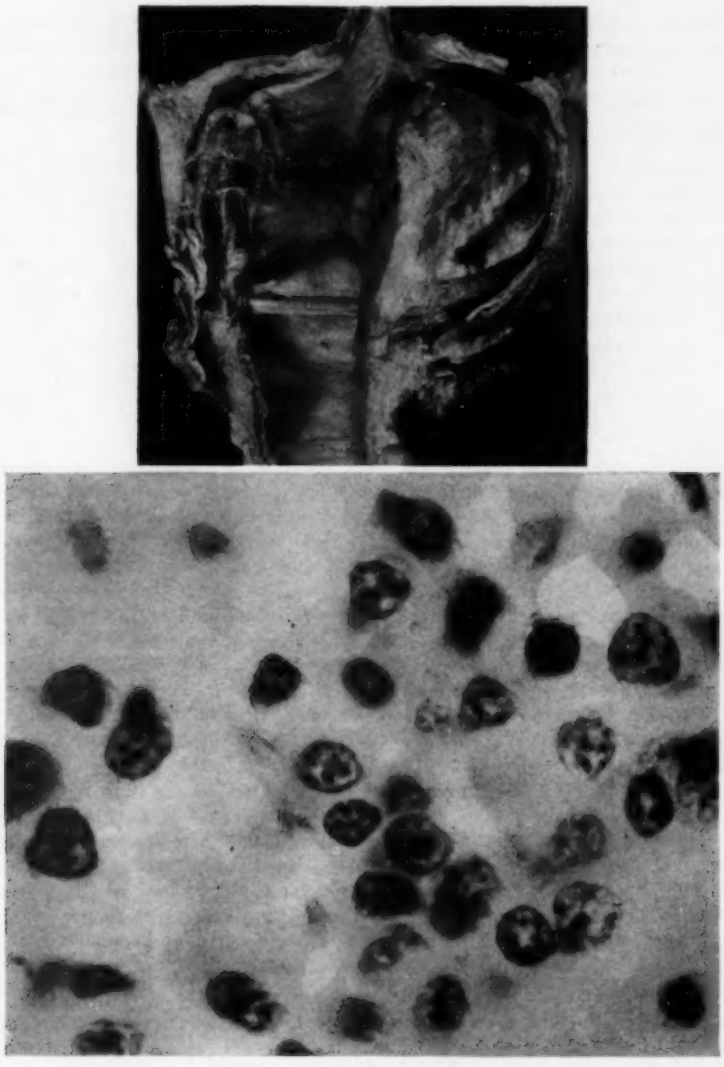
The individual cells varied in size and shape and in the size of their nuclei. The more mature cells were smaller and were typical of plasma cells, showing the eccentrically placed nucleus with the cartwheel arrangement of the chromatin. The large cells varied considerably in the size of both the nucleus and the cytoplasm. Many cells showed from one to four nuclei with darkly staining chromatin. While in some cells the chromatin was arranged somewhat imperfectly, the cells with two nuclei showed as a rule typical distribution. Occa-

From the departments of pathology and surgery of Tulane University School of Medicine.

1. Verebely, T. V.: Beitr. z. klin. Chir. **48**:614, 1906.
2. Gilmore, G. B.: Arch. Otolaryng. **34**:453, 1941.

sionally, "giant" cells were seen which had a large amount of grayish blue homogeneous cytoplasm and large dense nuclei. Many cells were in division. Little stroma was seen, but in places areas of hyalinization were present.

Sections through the center of ossification of the thyroid cartilage revealed marrow in which active hemopoiesis and an occasional megakaryocyte were evident. Numerous plasma cells crowded the cells of the erythroid and myeloid series. Spicules of bone could be seen.



The larynx and trachea have been opened by a posterior longitudinal incision. There is a mass attached to the right half of the cricoid cartilage. The right vocal cord is surrounded and pushed inward but not invaded. Below is a section through the tumor mass revealing diffuse infiltration by plasma cells in various stages of development.

COMMENT

A similar condition was described by Verebely. In his case small centers of ossification were present in both wings of the thyroid cartilage. The tumor arising at these points had obliterated the lumen of the larynx. At autopsy, in addition to the laryngeal involvement there was multiple myeloma of the ribs and vertebrae.

Gilmore reported the case of a white man, 69 years of age, with a subglottic mass at the level of the cricoid cartilage. The tumor, as well as the sternal marrow, was composed of large plasma cells. Circumscribed areas of bone destruction were present in the skull and the mandible. No follow-up examination or necropsy was done in this case.

SUMMARY

A case of multiple myeloma is reported in which a tumor apparently arose from an ossified thyroid cartilage containing red marrow. Serial sections through the tumor and the cartilage showed that the tumor took origin from this point. Only one similar case with postmortem observations has been described in the literature.

Notes and News

Appointments.—George J. Rukstinat has been appointed pathologist to the Presbyterian Hospital of Chicago.

Joseph Stasney has been made assistant professor of pathology in Jefferson Medical College, Philadelphia.

John R. Mohler, chief of the Bureau of Animal Industry of the U. S. Department of Agriculture, has retired after serving in the bureau for forty-six years. He will be succeeded by Arthur W. Miller, assistant chief of the bureau since 1928.

Enrique W. Lithgow, after working for two years under a fellowship of the Dazian Foundation in the department of pathology of Mount Sinai Hospital, New York, has returned to the Dominican Republic, where he will engage in pathologic practice and teaching.

Awards.—The William Osler Medal of the American Association of the History of Medicine has been awarded to George Edward Murphy, of the University of Pennsylvania School of Medicine, in recognition of his essay entitled "The Evolution of Our Knowledge of Rheumatic Fever."

The College of Physicians of Philadelphia has awarded the Alvarenga Prize to Ernest Carroll Faust, professor of medical parasitology and acting head of the department of tropical medicine of Tulane University, for his "outstanding contributions to our knowledge of parasitology and tropical medicine."

The Edward J. Ill Award of the Academy of Medicine of Northern New Jersey has been presented to Harrison S. Martland, of Newark, since 1925 chief medical examiner of Essex County. The inscription on the plaque reads "renowned pathologist, dynamic teacher, eminent civic benefactor."

Harvard Course in Legal Medicine.—A course in practical legal medicine for medical examiners, coroner's physicians and other qualified persons will be given at the Harvard Medical School, October 4 to 9 next. The number is limited to 15, and the fee is $25. Application should be made by October 1 to Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston. An open, all day conference on medicolegal topics will be held at the Mallory Institute of Pathology, Boston City Hospital, October 6.

Obituaries

JAMES EWING, M.D.
1866-1943

James Ewing was born in Pittsburgh on Dec. 25, 1866, the son of Thomas and Julia R. (Hufnagel) Ewing. He was educated in the public schools of Pittsburgh and by private tutors. He graduated from Amherst College in 1888 and then entered the College of Physicians and Surgeons of Columbia University, where he received his degree of Doctor of Medicine in 1891. At that time the medical curriculum covered but three years. On graduation he was undecided as to a future course and debated the advantages of a return to Pittsburgh rather than a career in New York medicine. Evidently the latter was more appealing for he applied for and secured an internship at the Roosevelt Hospital. This internship began in January of 1892, and in the intervening months between graduation and the commencement of the Roosevelt service he returned to Pittsburgh to serve on the surgical service of the Western Pennsylvania Hospital.

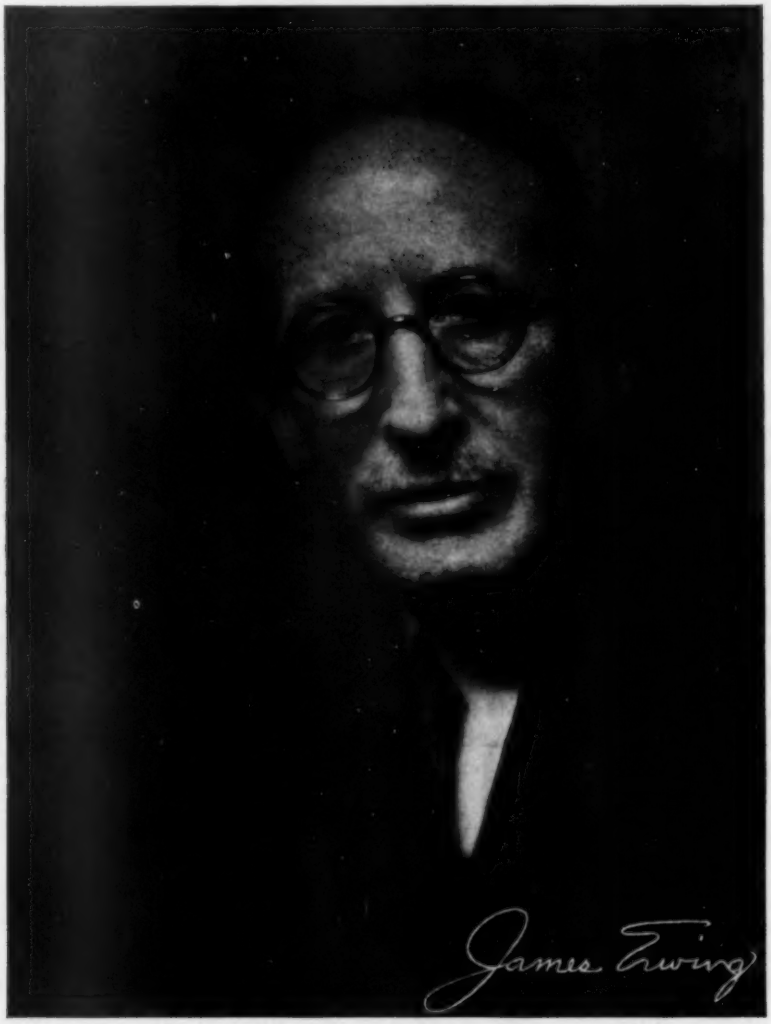
Ewing's real enthusiasm for medicine began at the Roosevelt Hospital. The attending physicians on the service were Dr. Francis Delafield and Dr. William H. Thompson. It was during this service with Delafield that Ewing first developed interests in the laboratories. Blood counting was in its infancy, and the first paper of the future pathologist concerned the behavior of the leukocytes in pneumonia. The pathologist to the hospital was Hodenpyl. Ewing was most successful in obtaining permissions for autopsies on his patients and liked to assist Hodenpyl in their performance. On completion of the Roosevelt internship, he served an obstetric service at Sloane Hospital for Women where he came under the watchful eye of T. Mitchell Prudden.

At first Ewing was attracted to the practice of medicine. He opened an office on West Fifty-Seventh Street with Dr. William S. Stone. He had few patients and concerned himself more largely with the laboratory. Ewing and Stone built a tennis court in the rear of their office building and the interest in tennis of their colleagues is said to have transformed the office into something more suggestive of a locker room in a country club than a place for the practice of a learned profession. A poker game conducted at the office served for diversion several evenings per week. Dr. Stone reports that Dr. Ewing was a poor player but a most enthusiastic one.

Prudden induced Ewing to become assistant in histology at the College of Physicians and Surgeons. This position he held for four years, after which time he became assistant in clinical pathology. In 1899 came a reorganization of the Cornell University Medical College, and Ewing was selected to head the department of pathology. The selection of a man of 33 to head an important post was a departure from accepted procedure but never caused regret. Evidently all was not smooth, for in later years Ewing asserted that no man should ever be called to a professorship of pathology without at least ten years' experience in the field.

His early papers deal with a variety of subjects and reveal no definite trend of endeavor. Circumstances evidently dictated his interests. The return of troops

from Cuba and Puerto Rico and the hospitalization of numerous malarial patients at Camp Wyckoff, Long Island, aroused Ewing's interest in malaria, and he contributed a number of studies on that disease while working among these troops. Only the refusal of the surgeon general to permit it dissuaded him from a similar study of yellow fever which might well have cut short his career at an early age. The exanthems attracted his attention, and he spent considerable effort in the



JAMES EWING, M.D.
1866-1943

study of smallpox at the "pest" hospital on North Brother Island. He published a well illustrated study on inclusion bodies about that time.

Dr. Ewing had married shortly after graduation. One of his greatest tragedies was the early death of his wife from toxemia of pregnancy. He never lost interest in that disease and for some years devoted his research to that field, contributing papers of lasting significance. After the death of his wife Dr. Ewing withdrew

from society. His social contacts became more limited, and there began a period of almost exclusive devotion to the laboratory and to his teaching. He practically lived in his laboratory, rarely leaving until late at night, reading and writing almost constantly. He had purchased a home in Westhampton, Long Island, where his son was brought up and which he visited on occasions. This remained his legal residence until his death. He loved Westhampton and his friends there, and one of his last testimonials was a dinner in his honor given by the citizens of Westhampton to their most honored fellow townsman.

Dr. Ewing's life was in many ways a difficult one. He had suffered from osteomyelitis of the femur during his college days and still had a discharging sinus during his medical years at Columbia. Only about the time of graduation was he able to discard a cane. During much of his intermediate life period he suffered from intractable trigeminal tic douloureux. In fact, his major work, "Neoplastic Diseases," was written during the remissions of that malady. After trials of more conservative treatment by injections of alcohol, Ewing submitted to surgical treatment in the hands of Harvey Cushing in 1926. The acute paroxysms were cured; a painful anesthesia persisted, and the corneal disturbances consequent on resection of the trigeminal nerve partially disabled him for the remainder of his life. Like many physicians he did not take kindly to surgical treatment, and it is still asserted that James Ewing was the worst patient ever to occupy a bed in the Peter Bent Brigham Hospital. The story is still told of his leaving the hospital, unannounced, with a pressure bandage still in place, to go to the home of the late Dr. Codman on the North shore and of Cushing's driving out to Codman's at midnight to force him to return to the hospital.

Dr. Ewing was a great teacher. His lectures were masterful demonstrations of the use of the English language, the product of his liberal education. He taught didactically but never to the point of exhaustion of his students. His lecture platform occupied his student laboratory. Dr. Ewing's method was to talk for some fifteen minutes and then to demonstrate the pathologic material of his talk with specimens and sections. He constantly emphasized clinical aspects of disease. At the end of the demonstration the lecture was resumed to be followed by another demonstration and so on until the period was finished. Thus fatigue was minimal and the association of the spoken word and the practical material of the day was emphasized. His last lectures were recorded stenographically (without his knowledge) by his last class and are published in a small volume.

He possessed the affection and esteem of his colleagues to a high degree. Those colleagues for whom he had the greatest affection were William Mecklenburg Polk, Lewis Atterbury Stimson (the father of the present Secretary of War), Graham Lusk, Stanley Benedict, Lewis A. Conner and William Elser, the "old guard" at Cornell. It was Polk who as dean first advised Ewing to devote his time to the development of the Memorial Hospital for the Treatment of Cancer and Allied Diseases as a novel project in the cancer field.

The last three decades of Dr. Ewing's life were inseparably linked with the development of the Memorial Hospital. The association was both natural and accidental. The Collis P. Huntington Fund for cancer research had become largely responsible for subsidizing research at the General Memorial Hospital. That hospital had been founded in 1884 as the New York Cancer Hospital. The corporate name of the institution had later been altered since it had been learned through experience that patients of those days objected to placing themselves in a hospital which bore the name "Cancer Hospital" because of the unfounded

opprobrium attaching to the disease cancer. The laboratory studies under the Huntington Fund were conducted in the old Loomis laboratories at Cornell, and as professor of pathology at Cornell Ewing quite naturally became associated with those studies. His name first appears in the report of the Collis P. Huntington Fund for 1908 in connection with research on infectious lymphosarcoma of dogs.

The accidental association with the Memorial Hospital came through the late James Douglas. James Douglas was a mining engineer with hereditary traits toward adventure. He was the son of a physician who through curious circumstances had come to this country but who made a rather precipitous exit due to the extreme interest manifested by a local judge in the source of cadavers which he as a surgeon secured for anatomic dissection. James Douglas became president of the Phelps Dodge Corporation. His interest in cancer came in a personal manner, and this contact with the disease aroused a lifelong desire to be of benefit to the patient with cancer. With the discovery of radium Mr. Douglas became intrigued with the possibilities of radium as a therapeutic agent and actually secured from the Curies a small supply of the new element. This he placed for trial in the wards of Guy's Hospital, London. He felt, however, that he should personally follow the experiments, and since his interests were American, he found it difficult to observe the conduct of work in London. Hence, he transferred his field of observation to the old New York Hospital for a time. He later decided that a general hospital was unsuited for his purpose, and casting about for an institution with a primary interest in cancer, he found the Memorial Hospital. With the late Dr. Howard Kelly he formed a corporation for the development, on a nonprofit basis, of American pitchblende deposits and hence secured additional radium. He furnished a source of funds for the research at the Memorial Hospital, quite considerable for those days, and built a laboratory. James Douglas and James Ewing became lifelong friends, and James Ewing became director of research and pathologist to the Memorial Hospital. It is interesting to learn that one of Ewing's few departures from the field of cancer was dictated by the illness of his friend Douglas. The latter had pernicious anemia. Ewing, cognizant of the extreme gastric atrophy of that disease, secured hog stomachs from the abattoir, froze and desiccated the mucosas and fed the product to his friend. He secured a favorable response, but neurologic changes were already marked and the outlook was hopeless. After the death of Douglas, Ewing manifested no further interest in pernicious anemia and continued his life work. He did not know, of course, that he was anticipating ventriculin. Nevertheless, this brief clinical experiment was an index of the capacity of Ewing to convert his knowledge of pathologic anatomy into practical therapeutics when the occasion arose.

This enthusiasm for practical therapeutics dominated Ewing's career at the Memorial Hospital. He could never dissociate pathology and research from treatment of disease and manifested a constant enthusiasm for clinical experiment. Whatever the future of radium or of radiotherapy may be, whether these methods grow in usefulness or become discarded, it must be admitted that Ewing insisted on their trial to the utmost. Many surgeons came to feel that he was opposed to surgery, that he disliked surgeons as a class and disapproved of their methods. The fact is that Ewing never discouraged surgical intervention once he became convinced that it offered the best possibility of cure or relief from suffering. He was most antagonistic to surgery for surgery's sake, to extensive surgical work in the face of incurable disease and to surgical intervention by surgeons who lacked understanding of the natural history of cancer and of the total inefficacy

of their ill planned procedures for interrupting this natural course. He was less vocal in the face of ill planned or ill conducted radiotherapy but justified it in the name of clinical experiment. Whatever eventually comes to radiotherapy, the radiotherapy of today owes Ewing a great debt.

The same is true of the pathology of tumors. The awakening of interest in the pathology of tumors in this country was surely influenced to a large extent by Ewing's book, "Neoplastic Diseases." Few pathologists had had the occasion or the interest to view the whole field of pathology of tumors and to explore its vastness from the point of view of practical natural history. Ewing's treatise stimulated the interest of both pathologists and clinicians in this field. Moreover, the existence of the Memorial Hospital, largely the shadow of James Ewing, stimulated the foundation and growth of other similar institutions and hence had a profound effect on the whole idea of special hospitals for the concentration of patients with cancer for laboratory and clinical experiment and for the teaching of men whose future could be devoted to the care of patients with cancer. Ewing was one of the founders of the American Society for the Control of Cancer and as president charted many of its policies. He was likewise intimately associated with the early development of the American Association for Cancer Research and assisted in organizing the first International Cancer Congress. For many years he served on the editorial board of the *Journal of Cancer Research,* founded by an early colleague at Cornell, Richard Weil. He was one of the original members of the National Advisory Cancer Council and likewise assisted in an advisory capacity in the establishment of the International Cancer Research Foundation. His influences were beneficent, and efforts at control were uniformly through persuasion rather than command. He was a profound hater of the type of person whom one might characterize as fundamentally a medical politician, a profession which he held in utmost contempt, even though he often sought excuse to condone the action of such persons.

James Ewing's later years brought many honors. He received the honorary degree of Doctor of Science from Amherst College, the University of Pittsburgh, Union University and the University of Rochester; the Doctorate of Laws from Kenyon and Western Reserve. He received the Clement Cleveland medal, the Caldwell medal and the distinguished service medal of the American Medical Association. He held the Charles Mickle fellowship from Toronto (Canada) and was John Scott medalist (Philadelphia). He was Officer of the Order of Leopold (Belgium) and Decorated Officer of the Order of the Southern Cross (Brazil). He was an honorary member of the Swedish Roentgen Society, the Academy of Medicine (Budapest) and the Philadelphia Pathological Society. The Faculty of Medicine of Santiago de Chile made him honorary professor and the University of San Marco of Lima, Peru, the oldest university of the western hemisphere, gave him an honorary degree when he was on a good will tour through the countries of South America. He was a member of the National Academy of Sciences, the New York Academy of Medicine, the American Association of Pathologists and Bacteriologists, the American Association for Cancer Research, the Association of American Physicians, the Harvey Society, the Society for Experimental Biology and Medicine, the American Roentgen Ray Society and the Society of American Medical Museums. He was the last recipient of the gold-headed cane of the American Association of Pathologists and Bacteriologists, which had descended from Ernst, through Theobald Smith, William H. Welch and Frank B. Mallory.

Dr. Ewing retired from his professorship of pathology at Cornell in 1932 and thenceforth devoted his full time to the Memorial Hospital. He retired from

the latter in 1940 at the age of 73. Even after retirement he manifested continued interest in medicine and in cancer. He visited his laboratory daily, worked when he found time, and when illness at last forced him to seek medical care it found him still at work, engaged in the preparation of a treatise on the medicolegal aspects of cancer. He died on May 16, 1943. More than a thousand people attended his last rites. Testimonials of affection and esteem came from all walks of life. It is not without significance that at these rites were on the one hand Mr. John D. Rockefeller, who made the present Memorial Hospital possible, and on the other the humble friends from Westhampton, because James Ewing was a friend of all men of good will.

FRED W. STEWART.

H. GIDEON WELLS, M.D., PH.D.
1875—1943

AN APPRECIATION

The passing of an eminent man after a long, productive and influential career calls for more than a casual inquiry into those qualities of soul and mind which contributed to his success. For just as pathologists are accustomed to summarize their necropsy records in the well known "anatomical diagnosis," so should we assemble the human attributes of life into a sort of "spiritual diagnosis" in order to recognize and understand those spiritual traits which may have determined the character of an unusual man.

H. Gideon Wells possessed to a remarkable degree some of the highest qualities of human kind: a brilliant mind, an extraordinarily friendly personality, youthful enthusiasm and boundless energy. Long will his students and associates recall with pleasure his buoyant nature, his quips and repartee whereby he vitalized the teaching of pathology and made of himself one of the great teachers of his time. Rarely does a teacher have an opportunity to come into contact with so many young medical students during their formative years and to instil into them a respect and love for a subject; his more than five thousand students throughout the world will not forget his influence on their early development. During a time when academic rewards went largely to those most active in research he strove constantly to attain a high standard of teaching; even in his later years he "prepared" for his classes with the conscientious and nervous zeal of a young instructor.

The following poem, "Why I Teach," found in his desk had obviously appealed to him; it seems fitting, therefore, to reproduce it here as a final testimonial to a great teacher.

Because I would be young in soul and mind
Though years must pass and age my life constrain
And I have found no way to lag behind
The fleeting years, save by the magic chain
That binds me, youthful, to the youth I love,
I teach.

Because I would be wise, and wisdom find
From millions gone before whose torch I pass,
Still burning bright to light the paths that wind
So steep and rugged, for each lad and lass
Slow climbing to the unrevealed above,
I teach.

Because in passing on the living flame,
That ever brighter burns the ages through,
I have done service that is worth the name
Can I but say "The flame of knowledge grew
A little brighter in the hands I taught,"
I teach.

Because I know that when life's end I reach
And thence pass through the gates so wide and deep
To what I do not know, save what men TEACH
That the remembrance of me will keep
Is what I've done; and what I have is naught,
I teach.

Louis Burton Woodward.

But it was not alone as a teacher that he excelled; his name stands forth in most of the important branches of pathology. He entered medicine well prepared for an investigative career in consequence of an exceptionally fine biochemical training under Chittenden and Mendel. When he became a pathologist, in 1897, Virchow's influence dominated pathology. Wells, by the utilization of chemical methods, initiated in this country a new attitude toward the study of disease. His early work on autolysis was followed by the important contributions to the subject of calcification, as described later in his Harvey Lecture. Soon he became interested in purine metabolism and its relationship to comparative biology and genetics. Enzyme reactions next engrossed him and directed his interests to immuno-

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chemistry. These studies all led, within a decade, to the publication, in 1907, of his book "Chemical Pathology." This book, the first of its kind, was truly monumental and has been a source of aid and inspiration to hundreds of pathologists during its passage through five editions.

It was during these early years, also, that he made his most significant contributions to immunology. One of these was his suggestion that the problems of immunology demanded the utilization of pure materials as surely as did those in other branches of chemistry. Thus, in 1908, when much of the confusion was attributable to the current immunologic methods of "mud-hole" chemistry, Wells said: "In developing the investigations of the chemistry of anaphylaxis, it seemed

desirable, first of all, to discard the heterogenous mixtures that have commonly been used in these studies, and, as the reaction is one concerning proteins, to work with the purest proteins obtainable that are suitable for the purpose." Through collaboration with his former teachers Osborne and Mendel he was able to secure the purified vegetable proteins with which he performed his important series of experiments on anaphylaxis.

These early evidences of his brilliant potentialities made it inevitable that soon he would suffer the usual academic fate and be obliged to assume greater administrative responsibility. It is not surprising, therefore, that when the Otho S. A. Sprague Memorial Institute was established on May 1, 1911, its young director should be H. Gideon Wells. At that time he was 36 years old and had published fifty scientific papers and one first class book.

From 1911 until his retirement from the University of Chicago in 1940 he directed the scientific activities of scores of workers in many diverse fields, interrupted only by a short interlude during World War I when he served with the American Red Cross Commission to Rumania. It was during the first year of existence of the Otho S. A. Sprague Memorial Institute that there came to him a young zoologist who wished to study hereditary aspects of cancer, and for thirty years Maud Slye was enabled to carry on her work because of his foresight and assistance. During these three decades he applied the resources of this institute to the study of experimental cancer, of the chemotherapy of tuberculosis, of edema and many other important problems. Although his program in the chemotherapy of tuberculosis did not achieve the good fortune of a Domaghk, the years of patient and systematic work by Wells, DeWitt, Corper, Long and others helped to eliminate many false concepts and thereby point the way to more promising methods of attack. The fruits of these years of significant research are now preserved in the twenty-five volumes of "Studies from the Otho S. A. Sprague Memorial Institute."

During all his active years he continued to accumulate a storehouse of medical information which he used in his own books and writings and which he also gladly shared. His card index file was immense, and as one student said, it was almost unique in that "H. G." knew everything that was in it and thus could make it available to others in time of need. As a matter of fact, his collector's instinct was seen also in his hobbies, as well as in his scientific proclivities; he once said that he would like to have been a paleontologist because there were so many interesting fossils to collect. These tendencies, fortunately, were expressed professionally in the pathologic museum which he assembled over a period of forty years; in its preparation he paid personal attention to the mounting of almost every specimen. And in his books he similarly assembled the outputs of the minds of countless workers in the fields of chemical pathology, immunology, oncology and infectious disease. But in addition to the instinct of a collector he had the unusual ability to discern significant contributions and thus to put each item into its proper place.

Of all his personal qualities perhaps his most consistent was that of loyalty to his friends and associates, particularly to those who had greatly influenced him. Two of these, Lafayette B. Mendel and Ludvig Hektoen, should be mentioned here because of their noteworthy influence on his life and character. He always referred to Mendel as his early inspiration and guide, and from him he got his love for chemistry. But Ludvig Hektoen meant more to him than did all others. He often alluded to Hektoen as his scientific father to whom he could always go for aid and comfort, certain that he would receive both. Toward his colleagues, assistants and associates he was always loyal; he had remarkably few prejudices

and exhibited few evidenecs of intolerance. He liked the world and the people in it, and the discerning ones reciprocated.

When he suffered his cardiac attack in 1932 his customary dynamic life had perforce to terminate, but this unhappy necessity failed to damp his sanguine spirit. He gave up golf and readjusted cheerfully to a slower tempo. A few years later, however, rapidly developing deafness dealt him the cruelest blow of all and took away much of his reserve of *joie de vivre*. In time, however, even this was compensated for, although the old sparkle was now gone. The final blow, coming insidiously in the form of carcinoma of the cecum, he met with fortitude and his usual jests. He prepared with courage and with cheerfulness for the operation from which he realized he would probably not recover. Death occurred suddenly on April 26, 1943. Surviving him are Mrs. Bertha R. Wells and Lieutenant Gideon R. Wells, M.C., United States Army.

Thus came to an end a life remarkable in many ways. Perhaps both because of his catholicity of mind and his zest for life, H. Gideon Wells achieved an unusually diversified record of attainment. In the thirty-five years between 1897 and 1932 he published, alone or in collaboration, three first class books and one hundred and ninety scientific papers, an average of more than five publications a year. These papers not only covered a wide range of medical topics; they were also good papers. During this time he taught pathology to generation after generation of medical students, administered the affairs of the Sprague Memorial Institute and of the department of pathology of the University of Chicago, lectured throughout the country, served as president of all of the pathologic societies of which he was a member, directed a Red Cross Commission to Rumania, read voluminously in many fields and still found time to win medals at the gun club and to become adept at golf, fishing and bridge. His temperament was indeed that of the bee which as it goes from flower to flower collects the nectar and leaves its imprint as well. As he went joyously through life tasting its sweets to the fullest, he, too, left his imprint on his students, in his books and in his other scientific contributions. And he surely left the world a better place because he had touched it.

PAUL R. CANNON.